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MUTAGENIC SCREENING OF THREE DYES FOR MARKER GRENADES IN THE SALMONELLA REVERSION ASSAY, THE L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA ASSAY, AND IN VIVO SISTER CHROMATID EXCHANGE IN MICE

Final Report

Prepared by

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Health Effects Research Laboratory
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Research Triangle Park, North Carolina 27711

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Project Officers: Mary C. Henry, Ph.D., CPT Gary M. Bratt, P.E., CIH

Performed by
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Two dyes, C.I. Solvent Yellow No. 33, and a C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were tested for mutagenicity in the Salmonella Reversion Assay, the L5178Y/TK+/- Mouse Lymphoma Assay, and for Sister Chromatid Exchange (SCE) in vivo in mice. The in vitro mutagenicity assays were performed both with and without exogenous activation provided by Aroclor induced rat liver S-9. A >99.9% pure sample of the yellow dye [2-(2-quinolyl)-1,3-indandione] was also tested with and without exogenous activation in the Salmonella
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Reversion Assay and the L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay. Neither C.I. Solvent Yellow No. 33 nor the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were positive in inducing in vivo SCE. All three dyes were tested in the standard plate incorporation test in seven strains TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98. The dyes were negative with and without exogenous activation in TA98, TA1535 and TA1538. One test with TA1537 was positive using the >99.9% pure yellow dye. All three dyes gave weakly positive results (less than a twofold increase) with S-9 in TA100. All three dyes were clearly positive in TA102 and TA104 both with and without S-9. All three dyes were found to induce mutation at the thymidine kinase locus in mouse lymphoma cells. Preliminary experiments (not financially supported under this IAG) indicate that the three dyes are clastogenic to mouse lymphoma cells.

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FOREWORD

All of the mutagenicity assays were performed in the Genetic Toxicology Division of the Health Effects Research Laboratory (HERL), US Environmental Protection Agency (USEPA), Research Triangle Park, NC. The Salmonella Reversion Assays were performed under the direction of Drs. Joellen Lewtas and Larry Claxton. The L5178Y/TK$^{+/-}$ Mouse Lymphoma Assays were performed under the direction of Dr. Martha Moore, and the in vivo Sister Chromatid Exchange assays were performed under the direction of Dr. James Allen. The purified yellow dye (>99.9% pure 2-(2-quinolyl)-1, 3-indandione) was supplied by Drs. Rogene Henderson and Roger McClellan, Inhalation Toxicology Research Institute, Lovelace Biomedical and Environmental Research Institute, Inc., Albuquerque, New Mexico.

EXECUTIVE SUMMARY

Dyes are used by the military in M18 marker signaling grenades. A number of organic dyes are pr··ently being evaluated for potential use in these grenades. In addi..on to engineering studies for their performance in the field, the US Army is concerned with evaluating any potential health hazards that might result from personal contact with the dyes in the industrial setting. A part of this testing is the analysis of potential genetic toxicity.

Three dyes, C.I. Solvent Yellow No. 33, a purified C.I. Solvent Yellow No. 33 [>99.9% 2-(2-quinolyl)-1,3-indandione], and a C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were tested for potential genotoxicity. All three dyes were tested for mutagenicity in the Salmonella Reversion Assay and the L5178Y/$TK^{+/-}$ Mouse Lymphoma Assay. The C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were also tested for sister chromatid exchange (SCE) induction in vivo in mice. The in vitro mutagenicity assays were performed both with and without exogenous activation provided by Aroclor induced rat liver S-9.

Neither the C.I. Solvent Yellow No. 33 nor the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were positive in inducing in vivo SCE. All three dyes were tested in the standard plate incorporation test using Salmonella typhimurium. Seven tester strains were used (TA98, TA100, TA1535, TA1537, TA1538, TA102 and TA104). The dyes were not mutagenic either with or without exogenous activation in TA98, TA1535 and TA1538. One test with TA1537 was positive using the pure yellow dye. All three dyes gave weakly positive results (less than a twofold increase) with S-9 activation in TA100. All three dyes were clearly positive in TA102 and TA104 both with and without S-9. All three dyes were found to induce mutation at the thymidine kinase locus in mouse lymphoma cells. Preliminary experiments (not financially supported under this IAG) indicate that the dyes are also clastogenic to mouse lymphoma cells.

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INTRODUCTION

Dyes are used by the military in M18 marker signaling grenades. A number of organic dyes are presently being evaluated for potential use in these grenades. In addition to engineering studies for their performance in the field, the US Army is concerned with evaluating any potential health hazards that might result from personal contact with the dyes in the industrial setting. A part of this testing is the analysis of potential genetic toxicity.

Three dyes, a yellow dye (C.I. Solvent Yellow No. 33), a purified yellow dye (purified C.I. Solvent Yellow No. 33) and a green-yellow dye which is a mixture of C.I. Solvent Yellow No. 33 and C.I. Solvent Green No. 3 were tested in this study. C.I. Solvent Yellow No. 33 is classified chemically as a quinoline. The principle color additive of the C.I. Solvent Yellow No. 33 is 2-(2-quinolyl)-1,3-indandione. and the additive of the C.I. Solvent Green No. 3 is 1-4-di-p-toluidino anthraquinone.

In preliminary tests, (unpublished results) different production lots (from those used in this study) of the C.I. Solvent Yellow No. 33 and C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were evaluated in the Salmonella Reversion Assay (using strains TA98, TA100, TA1535, TA1537 and TA1538) and found to be non-mutagenic. The L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay and in vivo Sister Chromatid exchange analysis were chosen to analyze more fully the genotoxic potential of the dyes. In the course of the study it was established that both dyes were clearly positive in the Mouse Lymphoma Assay. Since the lots used in the present studies were different from those originally tested in the Salmonella Reversion Assay, these new lots of the dyes were retested in this assay. In these studies two new strains (TA102 TA104) were utilized in addition to the five standard strains. In order to determine if the principle color additive in the yellow dye or one of the impurities was responsible for the mutagenic activity, arrangements were made to obtain a >99.9% pure sample of 2-(2-quinolyl) -1,3-indandione from the Lovelace Biomedical and Environmental Research Institute, Inc. (BRDL). This pure dye was tested both in the Mouse Lymphoma Assay and also in the Salmonella Reversion Assay (using all seven tester strains).

MATERIALS AND METHODS

Organic Dyes

The dyes tested were:

Yellow Dye - [C.I. Solvent Yellow No. 33, 2-(2-quinolyl)-1,3-indandione]

Yellow-Green Dye - [a mixture of C.I. Solvent Yellow No. 33 and C.I. Solvent Green No. 3 (1-4-di-p-toluidino anthraquinone)]

Purified Yellow Dye - [C.I. Solvent Yellow No. 33, >99.9% pure

2-(2-quinolyl)-1,3-indandione]

Chemicals

C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were supplied by BRDL. Each was analyzed by high presure liquid chromatography (HPLC; reverse phase column; gradient of 90:10 methanol:water to 100% methanol in 10 minutes; 1 ml/min flow rate; UV detection at 254 nm). C.I. Solvent Yellow No. 33 was 93.1% 2-(2-quinolyl)-1,3-indandione, <1.8% phthalic acid/anhydride and <0.4% quinaldine by weight. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture was 95.0% 2-(2'-quinolyl)-1,3-indandione and 1,4-di-p- toluidino anthraquinone (in a 1:2 ratio), <0.6% phthalic acid/ anhydride, 0.2% quinaldine, 0.1% p-toluidine and <0.1% quinazarin.

Purified yellow dye was prepared by recrystallizing C.I. Solvent Yellow No. 33 three times from ethyl acetate. HPLC analysis showed that the sum of unknown UV absorbing impurities (quantities based on peak heights relative to parent compound), and phthalic acid/anhydride and quinaldine (quantitated using standards) was <0.1% of the 2-(2'-quinolyl)-1,3-indandione present.

SALMONELLA REVERSION ASSAY

The procedures used were those of Ames, et al. (1975) with minor modifications. Modifications are included in the description that follows. For each sample, seven histidine-requiring strains were used. The strains used were TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98. The mechanisms by which each of these strains revert to prototrophy are fully discussed in other publications (Ames et al., 1975; Maron and Ames, 1983). In addition to these basic mechanisms, the reader should keep in mind the following salient points. These strains carry an rfa mutation which produces a deficiency in bacterial cell wall lipopolysaccharides and increases the cell's permeability to large molecules; the uvrB mutation which decreases genetic repair; the R-factor plasmid in strains TA98 and TA100 increases their sensitivity by participating in error-prone repair and causes a higher spontaneous mutation rate. The seven strains differ in the number of spontaneous revertants per plate generally found. Compounds which are known mutagens for the different strains, with and without activation, were included in each assay as positive controls. The retention of phenotypic characteristics were checked with each test by examining for histidine auxotrophy (lack of growth on histidine deficient medium), deep rough character (sensitivity to crystal violet on a disk), UV-repair deficiency (sensitivity to UV light), and the presence of the appropriate plasmid (resistance to ampicillin on a disk).

Frozen permanent cultures containing fresh nutrient broth cultures with dimethylsulfoxide (DMSO) were maintained at -80°C. A working source of these cultures was maintained on master plates. All strains were initially grown in nutrient (Difco) broth at 37°C for 16 hours.

Preparation of Rat Liver S-9 Mix

Male CD-1 (Fisher derived) rats weighing approximately 200 g were given a single intraperitoneal injection of Aroclor 1254 (Ar) in corn oil (200 mg/ml) at a dose of 50 mg/kg of body weight. One day prior to termination the animals were taken off food but provided water ad libitum. The livers were aseptically removed and washed in sterile cold 0.15 M KCl. All subsequent steps were performed at 0° to 4°C with cold sterile solutions and sterile glassware. The livers were minced with scissors in 0.15 M KCl (3 ml/g wet weight liver) and homogenized with a Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 min at 9,000 x g, the supernatant (S-9) decanted and stored in convenient aliquots at -80°C.

The S-9 is mixed with a cofactor solution containing 8µmol MgCl, 32 µmol KCl, 5 µmol glucose-6-phosphate and 4 µmol nicotinamide adenine dinucleotide in 100 µmol of sodium phosphate buffer, pH 7.4. The amount of S-9 used in the S-9 mix was between 0.05 and 0.1 ml S-9/ml cofactor solution.

Test Procedure

For revertant selection, minimal Vogel-Bonner medium E supplemented with 1.5 percent Difco bacto agar and 2 percent glucose was used for base agar layers. The top agar (0.6 percent Difco bacto agar, 0.5 percent NaCl) at 45°C was supplemented with minimal amounts of histidine and biotin, the bacterial broth culture ($1-2x10^9$ viable cells per ml) and the test material dissolved in DMSO (supplied sterile, spectrophotometric grade). For tests without activation, 0.5 ml of buffer was added instead of the S-9 mix to the top agar. The plates were incubated in the dark at 37°C for 72 hr. The plates were examined for background growth and the number of colonies per plate were counted using an Artek 880 automatic colony counter.

Preincubation was accomplished by incubating the bacteria, the compound and/or solvent, and the activation system (S-9) (when required) at 37°C in a water bath. The culture medium was the same as the overlay agar except that the melted agar was not added until the incubation was completed. The preincubation period was 30 minutes. All other aspects of the procedure were the same as the plate incorporation test.

Statistical Analyses

Statistical tests and computer programs used were those of Stead, et al. (1981). This model assumes revertant colony formation at any dose follows a Poisson process, while the mean number of revertants per plate is a nonlinear function of up to four parameters. The resultant system of nonlinear equations is solved using a modified Gauss-Newton iterative scheme to obtain maximum likelihood estimates of the model parameters. Significance of the key parameters was tested by fitting reduced models and using likelihood ratio tests.

The determination of positives was based on the following criteria:

- The data must not vary significantly from a Poisson distribution ($p > 0.01$).
- The data must be acceptable by the test of adequacy of fit of the the model ($p > 0.01$).
- The test for mutagenicity, the slope of the curve, must be significant ($p < 0.01$).
- At least a twofold increase must have occurred over spontaneous levels at one or more doses; otherwise, the response is recorded as weak and/or questionable
- All positive and negative controls must have given expected responses as compared to HERL, USEPA historical values and those published by Ames et al. (1975).
- Histidine cross-feeding and/or contamination must not have been shown to occur.

The modeling of the bioassay provides a valuable aid to the researcher; however, each curve was (and needs to be) examined individually in order to assure confidence in the apparent conclusions of the statistical process. For example, if the dose response data "fit" statistically a horizontal line (response vs. dose), the model will under some circumstances record a mutagenicity p-value less than 0.01; however, since the slope equals zero the response is negative.

The reader must also keep in mind that these particular tests were performed to maximize the chance of detecting a mutagenic response and not to provide comparative slope values. Examination of the data, therefore, shows that test doses were often adjusted due to results of a previous test. These adjustments obviously can shift results from a negative response to positive result (e.g. if a compound was initially tested at too low a dose response range) and may alter the slope value (e.g. providing more doses in the central portion of the dose-response curve).

The minimum testing requirements were as follows:

- A minimum of five doses at half-log intervals with the highest dose being highly toxic, as shown by background clearing and/or reduction in expected revertant counts per plate.
- Spontaneous and positive controls done at least in duplicate and providing the expected response as compared to HERL, USEPA historical values and those published by Ames et al. (1975).
- Positive controls (in duplicate) for the microsomal activation combination used are within normal ranges as compared to HERL, USEPA historical values and those published by Ames et al. (1975).
- These minimum criteria are carefully explained in other publications (Ames, 1975; de Serres and Shelby, 1979).

L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA ASSAY

The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture, C.I. Solvent Yellow No. 33 and the purified yellow dye were evaluated for mutagenicity in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay using the procedures of Clive and Spector, 1975, as amended by Clive et al., 1979, and Moore and Clive 1982. This in vitro mammalian system evaluates mutations affecting the thymidine kinase locus. This assay may be particularly useful in a test battery since the mutants quantitated can be divided, by colony size, into two distinct groups (small colony and large colony mutants). These two classes of mutants appear to reflect the relative clastogenic and mutagenic potential of the compound tested. Hozier et al. (1981, 1983) have shown that the majority of small colony mutants reflect chromosome damage affecting chromosome 11 (the location of the thymidine kinase gene), while large colony mutants appear to represent small scale, perhaps single gene damage.

Cell Line and Cell Maintanence

The TK$^{+/-}$-3.7.2C heterozygote of L5178Y mouse lymphoma cells (supplied by Dr. Donald Clive) was utilized. This cell line was routinely grown in supplemented Fischer's Medium for Leukemic Cells of Mice (see below). Cells were monitored daily (except for weekends)

for acceptable growth rates. For weekends, the cells were sufficiently diluted so that they would remain in log phase growth; weekend cell doubling times were always determined. Weekly, prior to use in the assay, cells were cleansed of spontaneous $TK^{-/-}$ cells by 24 hr growth in the presence of thymidine (3µg/ml), hypoxanthine (5µg/ml), methotrexate (0.1µg/ml) and glycine (7.5µg/ml) (THMG). This was followed by 24 hr growth in THG (THMG minus methotrexate) medium. Stock cells are stored in liquid nitrogen.

Media

$TK^{+/-}$ -3.7.2C cells were cultivated in Fischer's Medium for Leukemic Cells of Mice supplemented with 31 µg/ml penicillin (1650 units/mg), 50 µg/ml streptomycin sulfate, 0.1% Pluronic F68, 0.22 mg/ml sodium pyruvate(F_0P), and 10% horse serum to make $F_{10}P$. Medium was heat inactivated at 55° C for 45 minutes. Cells were cloned in the above described supplemented medium using 20% rather than 10% horse serum. In addition, 0.37% Noble agar was added to solidify the cloning medium for colony formation. The selective agent used for mutation at the TK locus was 1 µg/ml trifluorothymidine (TFT).

Preparation of Chemical Solutions

Concentrations were prepared on a weight per volume basis. DMSO was used as the solvent. A fresh stock of test material was used for each separate experiment.

Preparation of the Metabolic Activation System

Aroclor 1242-1254 induced rat liver S-9 was purchased from EG&G Mason Research Institute. Rats weighing 200-300 g were injected intraperitoneally with a 2:1 mixture of Aroclor 1242 and 1254 in corn oil (500 mg of total Aroclor/kg body weight). After 5 days the animals were sacrificed by CO_2 exclusion of air. They were totally immersed in a solution of Wescodyne for approximately three seconds and their heads quickly excised. The livers were removed and placed in preweighed beakers containing 0.25M sucrose. Livers were washed three times in 50-100 ml portions of cold 0.25M sucrose to yield 3 ml per gram of liver. Livers were minced and then homogenized in a teflon pestle tissue grinder. The homogenate was centrifuged at 9000 x g for

10 min at 4°C. The lipid layer was removed and discarded. The supernatant was pooled and aliquoted into sterile serum vials, and placed directly into liquid nitrogen vapor phase containers for storage prior to shipping. A sterility check and activity test for standard promutagens in the Salmonella Reversion Assay were performed prior to shipping.

Upon receipt the S-9 was stored at -70°C in a Revco freezer and tested for the ability to activate 2-acetylaminofluorene to mutagenic metabolites as based on induced mutant frequency in the standard mouse lymphoma assay.

The cofactor mix made just prior to addition was comprised of 600 mg of triphosphopyridine nucleotide (TPN) and 1125 mg of isocitric acid (trisodium salt, trihydrate) to 75 mls of F_0P (Fischer's medium supplemented but without horse serum). This solution was filter-sterilized, placed on ice and mixed with 25 ml of freshly thawed S-9 to form the S-9 mix. This mix was kept on ice until used.

Dose-Ranging Assay

The dose-ranging experiment consisted of increasing doses of the test compound to the level of highest solubility (in the DMSO solvent). One 50 ml Corning polypropylene tube seeded with 6×10^6 cells in 6.0 ml of medium with a reduced amount of serum (5% instead of 10%) was used for each dose. Four ml of serum-free Fischer's medium (F_0P) were added to each tube. The compound was dissolved in DMSO at 100 x the highest concentration to be tested. Sufficient solvent was added to each tube so that after addition of the test compound all tubes contained the same final solvent concentration. Normally 1% DMSO is the maximum used in this assay to deliver the test compound. Because of the low solubility of these dyes in DMSO the dose which could be delivered in 1% DMSO was significantly below the 1000 µg/ml normally used in a dose-ranging assay as the highest dose. Therefore, the amount of dye delivered in 2% and 3% (final concentration) DMSO and the appropriate solvent controls were also used. The test compound was added to each appropriately labelled tube, the tubes were then regassed with 5% CO_2-in-air and incubated in a roller drum at 37° C for 4 hr. Following the 4 hr exposure period the tubes are centrifuged for 10 min at 200 x g and the supernatant containing the test compound was discarded. The cells were then washed twice in 10 ml of $F_{10}P$ (2 X 10 minute centrifugations at 200 x g), and resuspended in 20 ml of fresh $F_{10}P$ to a final cell concentration of 3×10^5 cells/ml. The tubes were regassed with 5% CO_2-in-air and incubated in the roller drum at 37°C.

Cell counts were determined with a Coulter Counter Model ZBI at 24 hours after exposure to the compound. Relative growth (as compared to the negative control) was calculated for each culture.

Mutagenicity Assay

The doses chosen for the mutagenicity assay were based on the results of the dose-ranging study. Because the doses delivered in 3% DMSO showed no greater cell toxicity after 24 hr than the doses delivered in 2% DMSO, the 3% DMSO doses were not used. The dosing protocol is identical to that used in the dose-ranging study (i.e. cells were treated for four hours, washed and incubated at 37°C). Positive control compounds were tested with each experiment. Methyl methanesulfonate (MMS, 15 µg/ml) was used without exogenous activation, and 2-acetylaminofluorene (2-AAF, 40 µg/ml) with S-9 activation. Cell counts were determined with a Coulter Counter Model ZBI at 24 and 48 hrs. after exposure to the compound. Each culture was diluted daily to 2 X 10^5 cells/ml. At the end of 48 hrs the cells were cloned. Cloning allows for the selective growth and enumeration of mutant cells in a soft agar cloning medium (CM) and for the determination of cloning efficiency. Following dilution, the cells were allowed to mix for at least 30 minutes to minimize trauma. Fifteen ml of each culture was spun at 200 x g for 10 min and the supernatant decanted. Approximately 1-2 ml of $F_{10}P$ was added to each culture for resuspension of the cell pellet. The cell pellet was vigorously resuspended to ensure a single cell suspension and placed in 100 ml of CM to give a cell concentration of 3 X 10^4 cells/ml. The flasks were labelled with the appropriate culture number and selective agent to be used (TFT). The cells were allowed to acclimate for 30 minutes and then a 1:50 dilution was made. (1.0 ml was transferred from each culture to prelabelled flasks containing 50 ml of CM.) After mixing for 15 minutes, 1.0 ml from each 50-ml flask was transferred to 100 ml of CM and labelled with the culture number and "VC" (cell concentration = 6 cells/ml). The selective agent, 1 µg/ml TFT, was added to the flasks containing 3 X 10^4 cells/ml. Three petri plates per "TFT" and "VC" flask were poured, 33 ml per 100 mm petri plate. The plates were chilled at -20° C for 12 minutes, placed in a lucite box, sealed, and gassed with 5% CO_2-in-air or placed in a 5% CO_2 incubator. The boxes were incubated for 10-11 days at 37° C.

At the end of the incubation period the plates were scored for the number of colonies per plate using an Artek Colony Counter, Model 880. TFT-resistant colonies from selected cultures showing positive mutagenicity were sized by differential counts at periodic size discriminator settings. This information was expressed as histograms showing the relative proportions of small and large colony TFT-resistant mutants. This approach is a possible means of characterizing the type of mutagenic events occurring [i.e. single gene mutations (large colonies) or chromosomal aberrations affecting the TK and other genes (small colonies)].

Calculation of Mutant Frequency

The mutant frequency was calculated by dividing the total number of mutant colonies for each culture by the number of viable cells plated for the culture (as determined by the VC plates). The spontaneous mutant frequency (solvent control) was subtracted from the total mutant frequency to give the induced mutant frequency.

Criteria for the Evaluation of the Results

The following criteria (based on the statistical methods of Clive et al., 1979) must be met to designate the test compound as a definite positive:

1. One or more doses (from at least 2 separate assays) must show a significant increase (usually at least a doubling) over the background mutant frequency at reasonable (>10%) survival.
2. There must be a multi-point dose-related response at adequate (>10% survival) cytotoxicities.

If there is no significant increase of the mutant frequency over background and if the compound has been adequately tested (with and without metabolic activation, reasonably spaced doses, adequate cytotoxicity—sufficient doses in the 10-20% survival range) then the results will be interpreted as negative.

The minimum criteria for an acceptable assay are: (1) the plating efficiency of the solvent control is between 50 and 115%, (2) the spontaneous mutant frequency of the solvent control is less than 100×10^6 and (3) the positive controls show a definite positive response.

Method for Analysis of Gross Aberrations in L5178Y/TK$^{+/-}$ Mouse Lymphoma Cells

For the analysis of gross aberrations, samples were taken from the treated cells 24 hr after the midpoint of the 4 hr treatment period. Colcemid was added and cells treated with hypotonic KCl and fixed in acetic acid: methanol (1:4). Slides were made and cells stained with Wright's Stain. Metaphase spreads showing a near normal number of chromosomes were scored for aberrations.

IN VIVO SISTER CHROMATID EXCHANGE ANALYSIS IN MICE

Male C57BL/6 mice, 3-4 mos. old, were obtained from the Jackson Laboratory, Bar Harbor, Maine, and were acclimatized for at least 10 days after receipt. Animals were housed 5 per cage in an USEPA animal facility in laminar-flow rooms, with 15 cycles/hr of biocleaned air at 60-68% relative humidity. The room temperature was maintained at 68-70° F with a 12 hr light-dark cycle. Animals were fed lab chow (non-certified Purina) and water ad libitum.

Sister Chromatid Exchange (SCE) frequencies and cell replication kinetics were analyzed in mouse bone marrow cells after DNA labelling with 5-bromodeoxyuridine (BrdU; Sigma Chemical Company, St. Louis, Mo.). In vivo labelling was achieved with BrdU tablet methodology (Allen et al., 1978; McFee et al., 1983). Fifty mg BrdU tablets were prepared with a Parr Pellet Press and 0.178 in diameter punch and die (Parr Instrument Co., Moline, Il.) and coated over approximately 85% of the surface area with melted embedded paraffin (Fisher). Each experimental animal (weighing from 22 to 30 g) was implanted subcutaneously (lateral abdominal region) with a 50 mg BrdU tablet after brief anesthetization with Metofane (Pittman-Moore) inhalation.

Dye effectiveness to induce SCEs was determined by administering the test chemical as a single intraperitoneal injection (I.P.) ($\leq$0.2 ml volume) over a 3-4 point dose range, 3-4 mice per dose. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture was administered in 0.1 ml DMSO + 0.1 ml corn oil. The C.I. Solvent Yellow No. 33 was administered in 0.1 ml corn oil. The C.I. Solvent Yellow No. 33 was administered in 0.1 ml (per 30g) DMSO only. (Higher volumes of DMSO were determined in preliminary experiments to be toxic, as evidenced by animal death or inhibited marrow cell-cycling. While corn oil appeared to enhance the solubility of the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture, it reduced the solubility of C.I. Solvent Yellow No. 33.) The dye injections were given 1/2 hr after BrdU tablet implantation. Negative control animals

were those which received no injections, and those which were injected with the solvent only. Positive control mice were injected with 15 or 30 mg/kg cyclophosphamide (Mead-Johnson). Approximately 23 hr later, all control mice were injected I.P. with 0.6 mg/kg of colchicine (Sigma) in order to collect metaphases. Treated mice were injected with colchicine after an additional 3-4 hr since preliminary chemical injection trials had indicated that cell-cycle delays were occurring. Two hours after colchicine injection, animals were sacrificed by cervical dislocation, marrow cells were harvested and processed through hypotonic (0.075 M KCl) and fixative (3:1 methanol: glacial acetic acid) steps, and slides were prepared in accordance with standard cytogenetic methodology (Latt et al., 1981). Chromatid differential staining was achieved with the Fluorescence-plus-Giemsa (FPG) technique (Wolff and Perry, 1974; Goto et al., 1978). For each mouse SCE frequencies were analyzed in 30 randomly selected, well-differentiated second division metaphase cells which contained the diploid ± 2 chromosomal complement. Cell replication kinetics were also assessed in 200 marrow cells/ animal. The proportions of first (M_1), second (M_2) and third (M_3) division cells were determined from chromosome stain patterns.

Additional studies were performed to determine if: 1) the injected test dye was dispersing or remaining localized within the peritoneum, and 2) higher marrow cell SCE frequencies would result from giving injections over three consecutive days. Concerning the former studies, animals were examined at the time of marrow cell harvest for the appearance of internal localizations of dye particles. Peritoneal cells from control and high dose (35 mg/kg C.I. Solvent Yellow No. 33) animals were saline-washed from the peritoneum, pelleted and compared for evidence of dye crystals, and for viability (Trypan Blue Exclusion). Differential peritoneal cell counts (Wright's Stain) were also made. In the latter studies, concerned with multiple exposures to the test dye, all experimental design and cytogenetic features were the same as those described for the single exposure trials. The only protocol modification was the administration of 3 I.P. injections of the test material given 24 hr apart. BrdU tablet implantation was carried out just prior (1/2 hr) to the last injection, and cells harvested 24 - 28 hr later.

RESULTS AND DISCUSSION

SALMONELLA REVERSION ASSAY

The Salmonella bioassay is frequently used to screen substances for genotoxicity including potential carcinogenicity. The three dyes were tested in the standard plate incorporation assay using seven strains supplied by Dr. Bruce Ames. The seven strains used were TA100, TA102, TA104, TA1535, TA1537, TA1538, and TA98. In addition, the three dyes were also tested using TA100 in a preincubation assay. A summary of the results is in Tables 1A and 1B. The actual data and statistical analysis can be found in Appendix A. The results are very heterogeneous. Two of the strains that detect frame shift mutagens, namely TA98 and TA1538, gave negative responses both with and without exogenous metabolic activation for all three dyes. Although TA1537 gave a clearly positive response in only one test (with the purified yellow dye), all three dyes showed a consistent tendency for increased revertant numbers at the higher dose levels. The strain that responds almost exclusively to base pair substitution mutagens, TA1535, provided negative results both with and without exogenous metabolic activation. Even though negative results were associated with strains TA1535, TA1538, and TA98, the more non-specific strain TA100 gave a positive though weak response to all three dyes when S-9 was present. Without this mammalian metabolic activation, the TA100 results were negative. Strains TA102 and TA104 provided the clearest indication of the mutagenicity of these compounds. All three dyes were clearly positive both with and without S-9 when TA102 and TA104 were used. In contrast to the other five strains which detect mutations within GC sequences, these two strains require reversion to prototrophy within an AT rich region. These results may be typical of quinones (Maron and Ames, 1983). The use of the preincubation assay with TA100 did not provide a significant enough advantage to warrant its continued use with the other strains. Although a clear indication of mutagenicity was seen using three different strains, the three dyes were difficult to test primarily due to their solubilities. The samples began to precipitate out of solution at approximately the 100 µg per plate dose. This solubility problem not only narrowed the linear dose response range but also may have contributed to increased plate-to-plate variation. Within the bacterial assays, all three dyes gave very similar results. Since the purified yellow dye tended to yield a slope value greater than either of the other two dyes, the purified yellow dye is at least one of the major mutagenic components within the other dyes. Whether or not other mutagens are present within these dyes is not readily apparent from these data.

TABLE 1A MUTAGENICITY OF THREE ARMY DYES AS DETECTED BY SALMONELLA TYPHIMURIUM PLATE INCORPORATION AND PREINCUBATION (*) TESTS: (QUALITATIVE RESULTS)

Sample (Number)	Salmonella Typhimurium Strain: TA100	TA102	TA104	TA1535	TA1537	TA1538	TA98
	WITH ACTIVATION						
C.I. Solvent Green No. 3- C.I. Solvent Yellow No. 33 Mixture (BMGS-84-0001)	+	+	+	-	?[2]	-	-
	+	+	+	-	?[1]	-	-
	+*	+	...	...	...	-	...
C.I. Solvent Yellow No. 33 (BMGS-84-0002)	+	+	?[5]	-	-	-	-
	+	+	+	-	?	-	-
	+*	+	...	...	...	-	...
Purified Yellow (BMGS-84-0003)	+	+	?[5]	-	?	-	-
	+	+	+	-	+	-	-
	+*	+	...	...	...	...	...
	WITHOUT ACTIVATION						
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture (BMGS-84-0001)	?[1]	+	+	-	-	-[3]	-
	-	+	+	-	-	-	
	-*	+	...	...	...	-	...
C.I. Solvent Yellow No. 33 (BMGS-84-0002)	?[6]	?[4]	-	-	?	-	-
	-	+	+	-[2,3]	?	-	-
	-*	+	...	...	...	-	...
Purified Yellow (BMGS-84-0003)	-	?[4]	+	-	-	-	-
	-	+	+	-	?	-	-
	-*	+	...	...	...	...	...

Footnotes:
1 - Positive slope apparently due to single plate value.
2 - Positive slope apparently due to a single dose.
3 - Outlier was included in original calculation.
4 - Spontaneous control outside of normal range.
5 - Model did not converge adequately and results are borderline in nature.
* - Preincubation assay.
Results are recorded as follows: -, Negative; ?, questionable +, positive.

TABLE 1B MUTAGENICITY OF THREE ARMY DYES AS DETECTED BY SALMONELLA TYPHIMURIUM PLATE INCORPORATION AND PREINCUBATION (*) TESTS:

Sample (Number)	Salmonella Typhimurium Strain: TA100	TA102	TA104	TA1535	TA1537	TA1538	TA98
	----------WITH ACTIVATION----------						
C.I. Solvent Green No. 3- C.I. Solvent Yellow No. 33 Mixture (BMGS-84-0001)	0.2	6.9	6.1	Neg	?[2]	Neg	Neg
	0.7	3.1	4.2	Neg	?[1]	Neg	Neg
	1.2*	8.0	...	...	...	Neg	...
C.I. Solvent Yellow No. 33 (BMGS-84-0002)	1.8	2.6	?[5]	Neg	Neg	Neg	Neg
	1.5	5.6	2.9	Neg	?	Neg	Neg
	1.5*	5.8	...	...	...	Neg	...
Purified Yellow (BMGS-84-0003)	1.1	4.2	?[5]	Neg	?	Neg	Neg
	1.1	9.1	7.9	Neg	0.3	Neg	Neg
	2.1*	7.6	...	...	...	...	...
	----------WITHOUT ACTIVATION----------						
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture (BMGS-84-0001)	?[1]	5.0	2.4	Neg	Neg	Neg[3]	Neg
	Neg	3.3	2.4	Neg	Neg	Neg	Neg
	Neg*	5.1	...	...	...	Neg	...
C.I. Solvent Yellow No. 33 (BMGS-84-0002)	(?)[6]	?[4]	Neg	Neg	?	Neg	Neg
	Neg	1.6	3.5	Neg[2,3]	?	Neg	Neg
	Neg*	2.3	...	...	...	Neg	...
Purified Yellow (BMGS-84-0003)	Neg	?[4]	1.9	Neg	Neg	Neg	Neg
	Neg	4.8	1.5	Neg	?	Neg	Neg
	Neg*	6.0	...	...	...	...	...

Footnotes:

[1] - Positive slope apparently due to single plate value.
[2] - Positive slope apparently due to a single dose.
[3] - Outlier was included in original calculation.
[4] - Spontaneous control outside of normal range.
[5] - Model did not converge adequately and results are borderline in nature.
[6] - Value determined (1.2) appears to be an outlier since results could not be replicated.
* - Preincubation assay.

Results are recorded as follows: Neg, Negative; ?, questionable ; or as Revertants per µg substance per plate if positive. Each value represents an individual independent experiment.

L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay

The three dyes were tested both with and without exogenous metabolic activation in the L5178Y/TK$^{+/-}$ Mouse Lymphoma Assay. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture and the C.I. Solvent Yellow No. 33 were determined to be only slightly soluble in DMSO making testing over the normally prescribed (doses to 1000 µg/ml.) preliminary dose range impossible. A severely truncated dose range experiment was performed. Because of the solubility in DMSO problem and the concern that the dose delivered in 1% DMSO might not cause any toxicity, doses, and the appropriate solvent controls, delivered in 2% and 3% DMSO were also tested. (Normally the highest concentration of DMSO used for this assay is 1%). The results from this preliminary dose ranging experiment are found in Table 2. The actual mutagenesis experiments were performed using doses delivered in up to 2% DMSO. The pure yellow dye was found to be slightly more soluble in DMSO than the C.I. Solvent Yellow No. 33. Consequently, it was possible to test this compound at doses up to 50 µg/ml by delivering the dose in 1% DMSO. No dose-ranging study was necessary since the dose-range had already been established for the C.I. Solvent Yellow No. 33.

Table 3 shows the first experiment testing both the C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture with metabolic activation. The C.I. Solvent Yellow No. 33 is clearly positive while the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture gives only a (weak) positive response at 40 µg/ml. The cultures dosed with 6 µg/ml and above of the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture showed some precipitate following the treatment and after the first centrifugation. No precipitate was observed following the final resuspension. No precipitate was observed for the C.I. Solvent Yellow No. 33. In the repeat experiment (Table 4), the C.I. Solvent Yellow No. 33 is again clearly positive. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture gives a questionable response, with a possible positive at the high (40 µg/ml) dose. Precipitate was seen during the cell wash at doses above 16 µg/ml. Table 5 shows the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture tested over an expanded dose range. As in the previous tests, the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture shows a positive response only at the highest dose tested. Precipitate was observed during the cell wash in cultures treated at 15 µg/ml or more. The expanded dose range test for the C.I. Solvent Yellow No. 33 with metabolic activation (Table 6) confirms the positive response.

TABLE 2. DOSE RANGING EXPERIMENT FOR C.I. SOLVENT YELLOW NO. 33 AND C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN THE MOUSE LYMPHOMA MUTAGENICITY ASSAY

Concentration	24 hr. Relative Growth (%)
negative control	100
1% DMSO	100
2% DMSO	100
3% DMSO	100
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture	
2.07 µg/ml	90.1
4.14 µg/ml	92.9
8.30 µg/ml	84.4
12.4 µg/ml	70.2
16.6 µg/ml	66.2
20.7 µg/ml	75.6
41.4 µg/ml (2% DMSO)	74.0
62.1 µg/ml (3% DMSO)	85.0
C.I. Solvent Yellow No. 33	
1.94 µg/ml	81.2
3.90 µg/ml	83.5
7.8 µg/ml	74.9
11.6 µg/ml	74.7
15.5 µg/ml	69.8
19.4 µg/ml	76.9
38.8 µg/ml (2% DMSO)	82.8
58.2 µg/ml (3% DMSO)	72.2

TABLE 3. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO.33 MIXTURE AND C.I. SOLVENT YELLOW NO. 33 WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control (w/o S-9)	100.0	448	90	100.0	100.0	40.2	
Neg. Control	100.0	594	121	100.0	100.0	40.7	
Solvent Cont. (1% DMSO)	100.0	466	131	100.0	100.0	56.2	
Solvent Cont. (2% DMSO)	100.0	476	139	100.0	100.0	58.4	
Pos. Control (40 µg/ml 2 AAF)	29.5	435	815	93.3	27.5	374.7	318.5
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture							
2 µg/ml	112.4	484	185	103.8	116.7	76.4	20.2
6 µg/ml*	108.8	547	116	117.4	127.7	42.4	
12 µg/ml*	112.4	420	106	90.1	101.3	50.5	
16 µg/ml*	105.8	543	189	116.5	123.3	69.6	13.4
20 µg/ml*	109.3	515	159	110.4	120.7	61.8	5.6
40 µg/ml* (2% DMSO)	74.2	475	358	99.8	74.1	150.7	92.3
C.I. Solvent Yellow No.33							
2 µg/ml	110.8	546	142	117.1	129.7	52.0	
6 µg/ml	110.3	570	165	122.3	134.9	57.9	1.7
12 µg/ml	97.1	436	342	93.6	90.9	156.8	100.6
16 µg/ml	66.9	416	485	89.2	59.7	233.3	177.1
20 µg/ml	36.2	339	523	72.7	26.3	308.5	252.3
40 µg/ml (2% DMSO)	toxic						

*Showed some precipitate

TABLE 4. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE AND C.I. SOLVENT YELLOW NO. 33 WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control (w/o S-9)	100.0	435	106	100.0	100.0	48.7	
Neg. Control	100.0	435	119	100.0	100.0	54.7	
Solvent Cont. (1% DMSO)	100.0	407	145	100.0	100.0	71.3	
Solvent Cont. (2% DMSO)	100.0	456	157	100.0	100.0	68.9	
Pos. Control (40 µg/ml 2 AAF)	75.8	318	401	78.2	59.3	252.2	180.9
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture							
2 µg/ml	100.0	381	117	93.6	93.6	61.4	
6 µg/ml	121.9	498	143	122.4	149.2	57.4	
12 µg/ml	114.5	395	130	97.0	111.1	65.9	
16 µg/ml*	124.7	446	145	109.6	139.6	65.0	
20 µg/ml*	108.1	427	127	105.0	113.5	59.4	
40 µg/ml* (2% DMSO)	84.2	372	261	81.6	68.7	140.3	71.4
C.I. Solvent Yellow No. 33							
2 µg/ml	121.0	432	132	106.2	128.5	61.1	
6 µg/ml	124.2	445	145	109.4	135.9	65.1	
12 µg/ml	116.1	377	232	92.6	107.5	123.1	51.8
16 µg/ml	100.0	309	316	76.0	76.0	204.5	133.2
20 µg/ml	75.8	288	327	70.8	53.7	227.1	155.8
40 µg/ml (2% DMSO)	toxic						

*Showed some precipitate

TABLE 5. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Effic. (%)	Relative Total Growth (%)	Mutant Freq ($\times 10^6$)	Induced Mutant Freq ($\times 10^6$)
Neg. Control (w/o S-9)	100.0	378	92	100.0	100.0	48.7	
Neg. Control	100.0	301	89	100.0	100.0	59.1	
Solvent Cont. (1% DMSO)	100.0	374	93	100.0	100.0	49.8	
Solvent Cont. (2% DMSO)	100.0	344	79	100.0	100.0	46.0	
Pos. Control (40 µg/ml 2 AAF)	37.3	272	523	72.7	27.1	384.8	335.0
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture							
6 µg/ml	97.9	358	100	95.8	93.8	55.8	6.0
8 µg/ml	98.3	365	108	97.6	95.9	59.2	9.4
9 µg/ml	99.3	336	109	89.9	89.3	64.9	15.1
10 µg/ml	94.2	386	135	103.2	97.2	70.0	20.2
11 µg/ml	83.0	329	96	87.9	71.9	58.4	8.6
12 µg/ml	87.3	364	105	97.4	85.1	57.7	7.9
13 µg/ml	86.8	347	117	92.8	80.6	67.5	17.7
14 µg/ml	95.1	319	103	85.4	81.2	64.5	14.7
15 µg/ml*	91.5	357	107	95.5	87.4	60.0	10.2
16 µg/ml*	89.1	320	115	85.5	76.2	71.9	22.1
17 µg/ml*	90.2	334	118	89.4	80.6	70.6	20.8
18 µg/ml*	82.7	304	97	81.4	67.3	63.8	14.0
19 µg/ml*	85.8	309	101	82.7	70.9	65.4	15.6
20 µg/ml*	84.3	319	138	85.4	72.0	86.5	36.7
40 µg/ml* (2% DMSO)	42.3	248	340	66.3	27.8	274.4	228.4

*Showed some precipitate

TABLE 6. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT YELLOW NO. 33 WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control (w/o S-9)	100.0	326	90	100.0	100.0	55.2	
Neg. Cont.	100.0	359	110	100.0	100.0	61.3	
Solvent Cont. (1% DMSO)	100.0	304	62	100.0	100.0	40.8	
Pos. Control (40 µg/ml 2 AAF)	52.3	230	426	75.5	39.5	370.8	330.0
C.I. Solvent Yellow No. 33							
2 µg/ml	114.1	244	108	80.3	91.6	88.4	47.6
6 µg/ml	102.1	228	107	75.0	76.5	93.9	53.1
8 µg/ml	102.1	214	128	70.4	71.9	119.5	78.7
9 µg/ml	86.4	272	(no TFT)	89.3	77.2	-	-
10 µg/ml	90.6	281	107	92.3	83.6	76.2	35.4
11 µg/ml	85.0	249	151	81.8	69.5	121.3	80.5
12 µg/ml	78.8	225	198	74.0	58.3	176.0	135.2
13 µg/ml	66.2	261	238	85.8	56.8	182.4	141.6
14 µg/ml	70.3	281	196	92.3	64.9	139.6	98.8
15 µg/ml	66.0	179	201	58.8	38.8	224.8	184.0
16 µg/ml	52.9	255	162	83.8	44.3	127.1	86.3
17 µg/ml	57.9	209	235	68.6	39.7	225.1	184.3
18 µg/ml	45.6	169	180	55.6	25.4	212.8	172.0
19 µg/ml	48.8	235	275	77.3	37.7	233.8	193.0
20 µg/ml	39.4	220	173	72.4	28.5	157.1	116.3

The first test of the two dyes without exogenous metabolic activation is shown in Table 7. Both the C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were clearly positive. Green precipitate was observed during the cell wash at the 20 and 40 µg/ml doses of the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture. The repeat experiments (Tables 8 and 9) confirmed the positive response of both dyes without exogenous activation. Precipitate was visible in the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture treated cultures at doses above 8 µg/ml.

Both the C.I. Solvent Yellow No. 33 and the C.I. Solvent Green No. 3 C.I. Solvent Yellow No. 33 mixture give higher mutagenic activity without exogenous activation (Tables 7-9) than with activation (Tables 3-6). Both dyes are positive without metabolic activation at doses which do not show a precipitate. The C.I. Solvent Yellow No. 33 dye is also mutagenic with S-9 activation. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture is not mutagenic at doses showing no precipitate when S-9 activation is added to the system.

The purified yellow dye was also tested both with and without activation. The results are shown in Tables 10-13. As with the C.I. Solvent Yellow No. 33, the results are clearly positive. The response with exogenous activation is much weaker (Tables 10 and 11) than the response without activation (Tables 12 and 13).

It should be noted that this mutagenicity data obtained using the mouse lymphoma assay does not in all cases show a clear increasing dose-response relationship with increasing dose. This does not negate the positive nature of the data. Compounds which have solubility problems (i.e. are tested at doses near the limit of their solubility) tend to give plateau type dose-response curves similar to those observed in these studies. In addition it is not unusual or surprising that doses as close together as those used for these studies yield mutant frequencies which do not increase with each dose. In fact the closely spaced doses can almost be considered as replicates.

An analysis of the colony size distribution of the TFT-resistant mutants indicates that all three dyes produce significant proportions of small colony mutants (Figures 1-3, data with exogeneous activation not shown). This would predict that these dyes might also be clastogenic as well as mutagenic. To evaluate this possibility, gross aberration analysis of $TK^{+/-}$ mouse lymphoma cells treated with the dyes was performed. (This aspect of the research was not a part of

TABLE 7. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE AND C.I. SOLVENT YELLOW NO. 33 WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control	100.0	384	119	100.0	100.0	62.0	
Solvent Cont. (1% DMSO)	100.0	519	147	100.0	100.0	56.6	
Solvent Cont. (2% DMSO)	100.0	588	137	100.0	100.0	46.6	
Pos. Control (15 µg/ml MMS)	57.2	257	683	49.5	28.3	531.9	469.9
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture							
2 µg/ml	90.1	454	177	87.5	78.8	77.9	21.3
6 µg/ml	77.5	286	422	55.0	42.6	295.5	238.9
12 µg/ml	65.7	253	561	48.8	32.1	443.1	386.5
16 µg/ml	68.6	322	658	62.1	42.6	408.4	351.8
20 µg/ml*	59.2	253	473	48.8	28.9	373.6	317.0
40 µg/ml* (2% DMSO)	65.3	83	158	14.1	9.2	381.6	335.0
C.I. Solvent Yellow No. 33							
2 µg/ml	80.6	275	464	52.9	42.6	337.7	281.1
6 µg/ml	70.2	250	498	48.2	33.8	398.1	341.5
12 µg/ml	67.6	241	397	46.5	31.4	329.2	272.6
16 µg/ml	66.5	235	441	45.3	30.1	375.0	318.4
20 µg/ml	61.4	244	510	47.0	28.9	417.7	361.1
40 µg/ml (2% DMSO)	57.8	244	596	41.5	24.0	488.1	441.5

*Showed some precipitate

TABLE 8. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control	100.0	433	73	100.0	100.0	33.7	
Solvent Cont. (1% DMSO)	100.0	422	110	100.0	100.0	52.1	
Pos. Control (15 µg/ml MMS)	53.0	170	763	40.2	18.8	898.7	865.0
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture							
2 µg/ml	92.9	411	129	97.4	90.5	62.7	10.6
6 µg/ml	58.0	235	408	55.8	32.3	347.0	294.9
8 µg/ml	70.4	285	(no TFT)	67.6	47.6		
9 µg/ml*	70.8	253	473	60.0	42.5	373.6	321.5
10 µg/ml*	65.7	204	542	48.4	31.8	531.4	479.3
11 µg/ml*	60.9	248	611	58.7	35.7	493.1	441.0
12 µg/ml*	61.7	321	504	76.1	47.0	314.0	261.9
13 µg/ml*	58.4	248	546	58.7	34.3	440.7	388.6
14 µg/ml*	56.5	254	582	60.2	34.0	458.6	406.5
15 µg/ml*	65.3	256	587	60.7	39.7	458.2	406.1
16 µg/ml*	62.8	227	512	53.8	33.8	451.5	399.4
17 µg/ml*	63.0	268	645	63.6	40.0	481.0	428.9
18 µg/ml*	59.8	256	575	60.7	36.3	448.9	396.8
19 µg/ml*	57.4	262	611	62.2	35.7	466.0	413.9
20 µg/ml*	62.1	245	699	58.0	36.0	571.1	519.0

*Showed some precipitate

TABLE 9. MOUSE LYMPHOMA ASSAY OF C.I. SOLVENT YELLOW NO. 33 WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control	100.0	476	116	100.0	100.0	48.8	
Solvent Cont. (1% DMSO)	100.0	540	134	100.0	100.0	49.6	
Solvent Cont. (2% DMSO)	100.0	514	132	100.0	100.0	51.3	
Pos. Control (15 µg/ml MMS)	69.8	230	942	42.5	29.7	819.8	770.2
C.I. Solvent Yellow No. 33							
2 µg/ml	77.6	257	545	47.6	36.9	424.4	374.8
6 µg/ml	58.4	193	739	35.8	20.9	765.0	715.4
8 µg/ml	59.6	225	773	41.7	24.9	687.1	637.5
9 µg/ml	56.6	258	725	47.8	27.0	562.0	512.4
10 µg/ml	68.6	185	674	34.2	23.5	729.4	679.8
11 µg/ml	59.1	202	792	37.4	22.1	783.4	733.8
12 µg/ml	47.7	239	654	44.2	21.1	547.7	498.1
13 µg/ml	61.0	212	767	39.2	23.9	724.3	674.7
14 µg/ml	55.7	220	737	40.8	22.7	669.4	619.8
15 µg/ml	61.7	225	697	41.7	25.7	619.6	570.0
16 µg/ml	62.0	276	796	51.1	31.7	576.8	527.2
17 µg/ml	58.0	192	674	35.6	20.6	702.1	652.5
18 µg/ml	53.5	187	624	34.7	18.6	666.7	617.1
19 µg/ml	64.4	219	657	40.6	26.1	600.0	550.4
20 µg/ml	57.7	241	636	44.7	25.8	527.4	477.8
40 µg/ml (2% DMSO)	41.8	157	812	30.6	12.8	1033.1	981.8

TABLE 10. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control	100.0	363	67	100.0	100.0	36.90	
Neg. Control (w/o S-9)	100.0	334	79	100.0	100.0	47.40	
Solvent Cont.	100.0	294	83	100.0	100.0	56.50	
Solvent Cont. (w/o S-9)	100.0	263	70	100.0	100.0	53.30	
Pos. Control (40 µg/ml 2AAF)	74.2	227	219	77.1	57.2	193.12	136.66
Purified Yellow Dye							
5 µg/ml	95.3	278	62	94.5	90.0	44.60	
10 µg/ml	78.3	247	123	84.1	65.8	99.50	43.0
12 µg/ml	68.9	248	180	84.3	58.1	145.30	88.8
14 µg/ml	45.5	273	232	92.9	42.2	170.00	113.5
16 µg/ml	52.6	243	186	82.6	43.5	153.10	96.6
18 µg/ml	43.2	227	197	77.1	33.3	173.70	117.2
20 µg/ml	51.8	242	142	82.2	42.6	117.40	60.9
22 µg/ml	34.8	274	203	93.1	33.4	148.40	91.9
24 µg/ml	45.7	197	188	66.9	30.6	191.10	134.6

TABLE 11. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE WITH METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control	100.0	420	113	100.0	100.0	53.8	
Neg. Control (w/out S-9)	100.0	508	178	100.0	100.0	70.1	
Solvent Cont.	100.0	457	82	100.0	100.0	35.9	
Solvent Cont. (w/out S-9)	100.0	475	205	100.0	100.0	86.3	
Pos. Control (40 µg/ml 2AAF)	16.4	320	772	69.9	11.5	482.8	446.9
Purified Yellow Dye							
2.5 µg/ml	92.9	458	113	100.1	92.9	49.4	13.5
5 µg/ml	93.1	508	105	111.0	103.3	41.4	5.5
10 µg/ml	92.4	415	194	90.8	83.9	93.4	57.5
12 µg/ml	75.1	430	251	93.9	70.6	116.9	81.0
14 µg/ml	73.3	393	188	86.0	63.0	95.7	59.8
16 µg/ml	54.4	462	286	101.0	55.0	123.8	87.9
18 µg/ml	41.3	423	279	92.5	38.2	131.9	96.0
20 µg/ml	60.1	515	310	112.6	67.7	120.4	84.5
22 µg/ml	30.7	497	405	108.6	33.4	163.0	127.1
24 µg/ml	27.2	487	446	106.6	29.0	183.1	147.2

TABLE 12. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Effic. (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control	100.0	447	90	100.0	100.0	40.3	
Solvent Cont.	100.0	471	104	100.0	100.0	44.2	
Pos. Control (15 µg/ml MMS)	61.1	237	645	53.0	32.4	544.3	504.0
Purified Yellow Dye							
0.1 µg/ml	110.9	503	103	106.8	118.4	41.0	
0.5 µg/ml	107.9	372	144	79.0	85.2	77.4	33.2
2.5 µg/ml	60.7	262	683	55.5	33.6	525.9	481.7
5 µg/ml	50.2	200	493	42.4	21.3	493.5	449.3
10 µg/ml	33.3	130	542	27.5	9.2	836.4	792.2
20 µg/ml	30.9	120	566	25.5	7.9	943.3	899.1
30 µg/ml	53.3	224	476	47.5	25.3	425.4	381.2
40 µg/ml	57.5	239	506	50.7	29.2	423.8	379.6
50 µg/ml	53.8	181	353	38.3	20.6	390.9	346.7

TABLE 13. MOUSE LYMPHOMA ASSAY OF PURIFIED YELLOW DYE WITHOUT METABOLIC ACTIVATION

Concentration	Relative Suspension Growth (%)	Total Viable Clones	Total Mutant Clones	Relative Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Freq ($x10^6$)	Induced Mutant Freq ($x10^6$)
Neg. Control	100.0	299	116	100.0	100.0	77.6	
Solvent Cont.	100.0	326	150	100.0	100.0	92.1	
Positive Control (15 µg/ml MMS)	74.4	183	289	61.2	45.6	315.8	238.2
Purified Yellow Dye							
0.1 µg/ml	120.1	323	116	98.9	118.8	72.0	
0.5 µg/ml	87.4	250	148	76.8	67.1	118.3	26.2
1.0 µg/ml	54.7	168	198	51.6	28.2	235.7	143.6
2.5 µg/ml	71.5	203	239	62.2	44.5	235.7	143.6
5 µg/ml	62.9	153	212	46.9	29.5	277.1	185.0
10 µg/ml	61.4	151	263	46.4	28.5	347.9	255.8
20 µg/ml	52.9	158	258	48.4	25.6	326.9	234.8
30 µg/ml	49.6	134	212	41.1	20.4	316.9	224.8
40 µg/ml	57.3	142	203	43.6	25.0	385.5	293.4
50 µg/ml	43.8	127	222	39.0	17.8	349.1	257.0

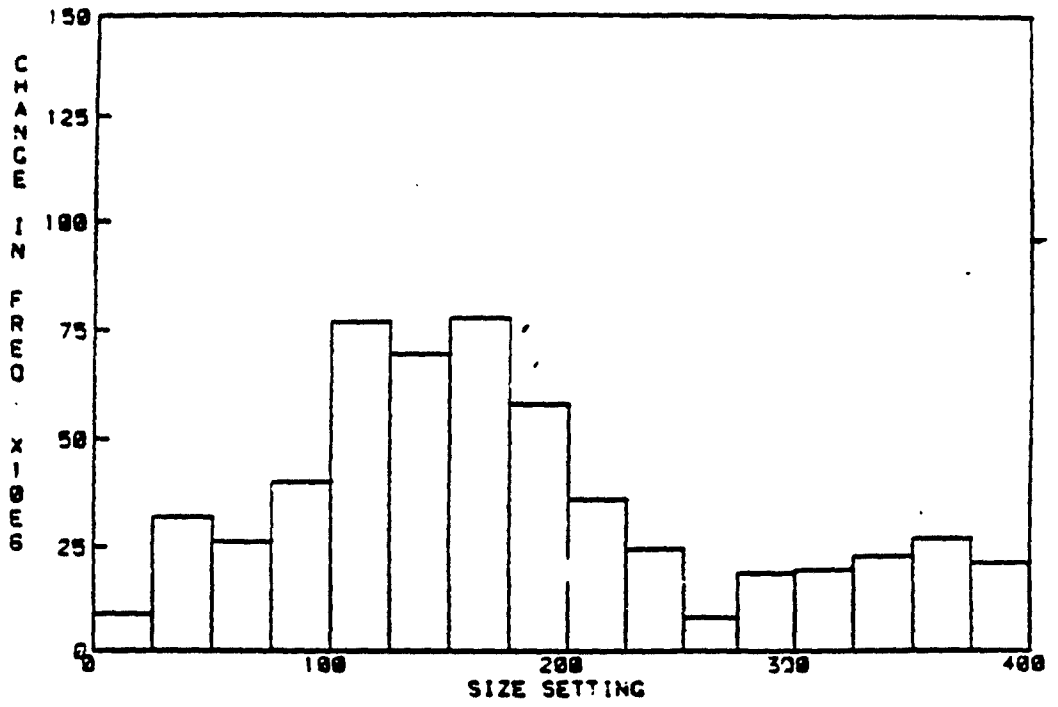

Figure 1: Relative size distribution of TFT-resistant mutants following treatment with 20 µg/ml of C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33, without exogenous metabolic activation. The small colonies are shown in the left peak; the large colonies in the right peak.

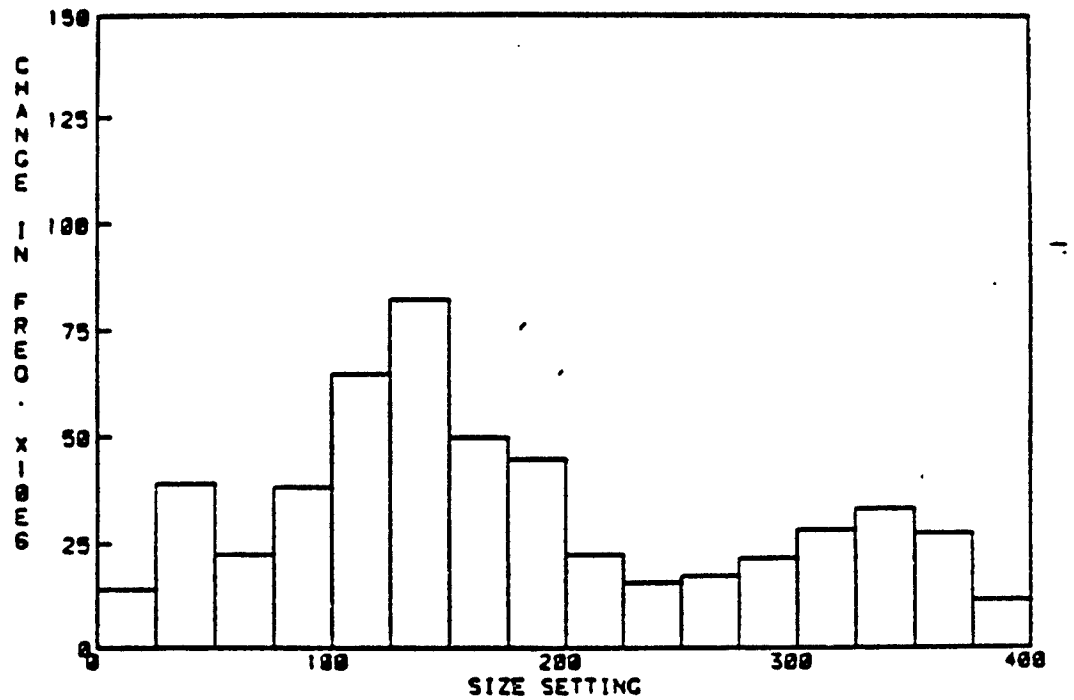

Figure 2: Relative size distribution of TFT-resistant mutants following treatment with 20 µg/ml of C.I. Solvent Yellow No. 33, without exogenous metabolic activation. The small colonies are shown in the left peak; the large colonies in the right peak.

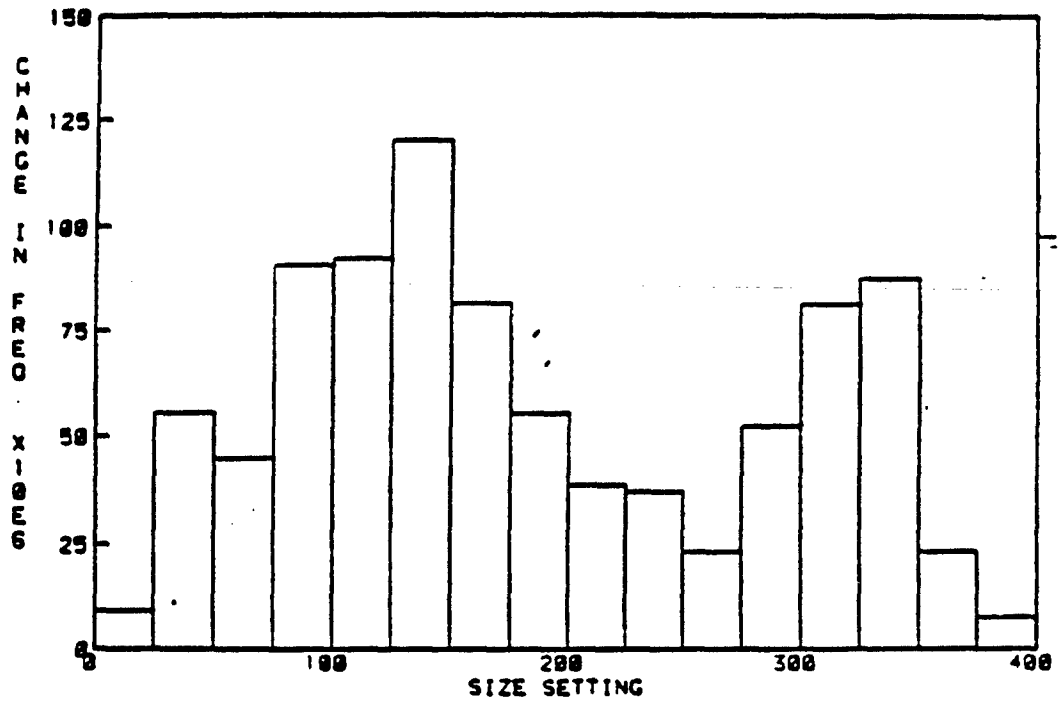

Figure 3: Relative size distribution of TFT-resistant mutants following treatment with 10 µg/ml of >99.9% pure yellow dye, without exogenous metabolic activation. The small colonies are shown in the left peak; the large colonies in the right peak.

the work requested by the US Army. It is included because of its significance and usefulness in the evaluation of these dyes for potential human health hazard.) All three dyes were found to be clastogenic (Figures 4-6) to these mouse lymphoma cells. It should be noted that 100 cells/dose were analyzed in Figures 4 an 5 while 50 cells/dose were analyzed for Figure 6. Chromosome breaks, translocations and chromosome deletions were induced by the dyes.

In Vivo Sister Chromatid Exchange Analysis in Mice

The results from analyses of marrow cell SCEs and cell kinetics are presented in Tables 14 thru 19. Individual animal mean SCE values and relative proportions of metaphase cells at the first, second and third division after BrdU and a single C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture exposure are provided in Table 14. Data for each exposure group are summarized in Table 15. Cyclophosphamide was clearly effective as a positive control. SCE frequencies were 7-8 times higher than negative control values, and significantly higher numbers of first division cells (with lower numbers of second and third division cells) evidenced a cytotoxic effect. However, there was no increase in SCE frequency at any dose due to exposure to the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture. A greater prevalence of third division cells sometimes noted in the dye treatment group was likely caused by the later times of cell harvest. The C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture was observed to be dissolved in solution at the 10 and 20 mg/kg doses, and precipitated out of solution at the 40 mg/kg dose.

Data similarly tabulated for single intraperitoneal exposure trials with the C.I. Solvent Yellow No. 33 are presented in Tables 16 and 17. This dye was also ineffective in inducing SCEs. Cyclophosphamide clearly induced SCEs and slowed cell-cycling; however, no such effects were observed after exposure to the C.I. Solvent Yellow No. 33. SCE levels were not significantly different from control levels, and higher numbers of third division cells (lower numbers of first division cells) probably were a reflection of later cell harvest times. The C.I. Solvent Yellow No. 33 was observed to be in solution at the 5 and 15 mg/kg doses and precipitated out of solution at the 25 and 35 mg/kg doses.

Similar negative results were obtained after exposure to multiple injections of the C.I. Solvent Yellow No. 33 (Tables 18 and 19). Cyclophosphamide was effective; however, the dye-treated animals generally showed no greater SCE frequencies or cytotoxicity. One

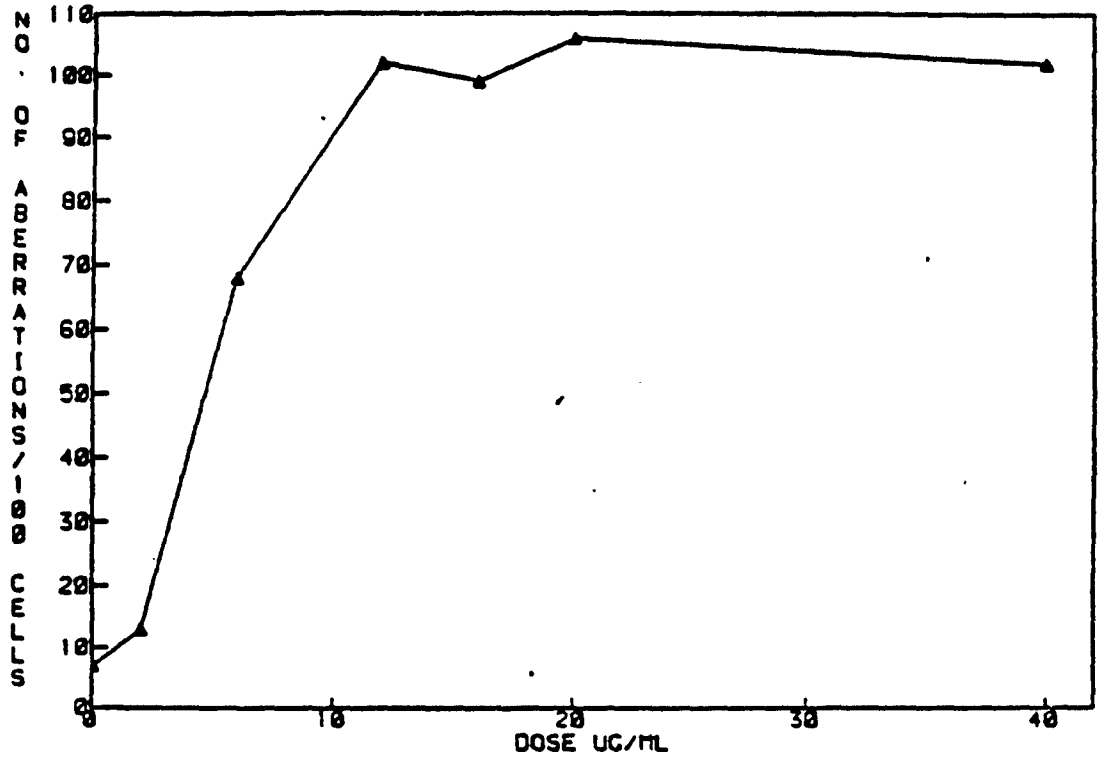

Figure 4: Gross aberration frequency in L5178Y/TK$^{+/-}$ Mouse Lymphoma cells following treatment with C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture, without exogenous metabolic activation.

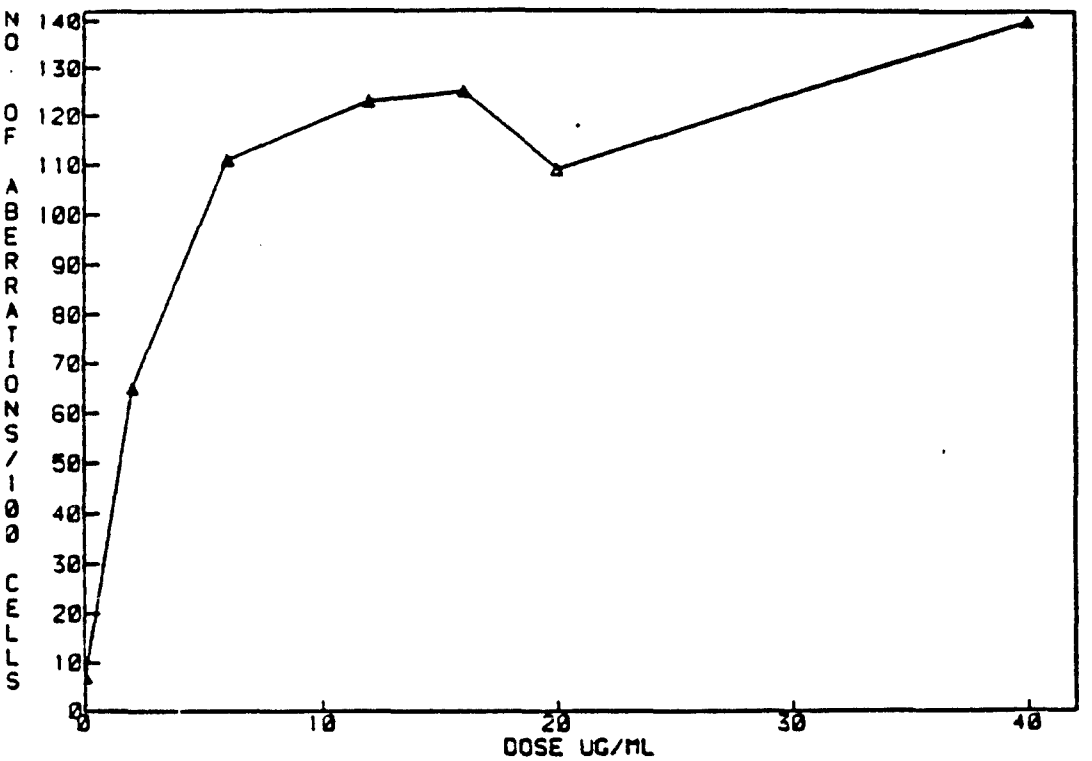

Figure 5: Gross aberration frequency in L5178Y/TK$^{+/-}$ Mouse Lymphoma cells following treatment with C.I. Solvent Yellow No. 33, without exogenous metabolic activation.

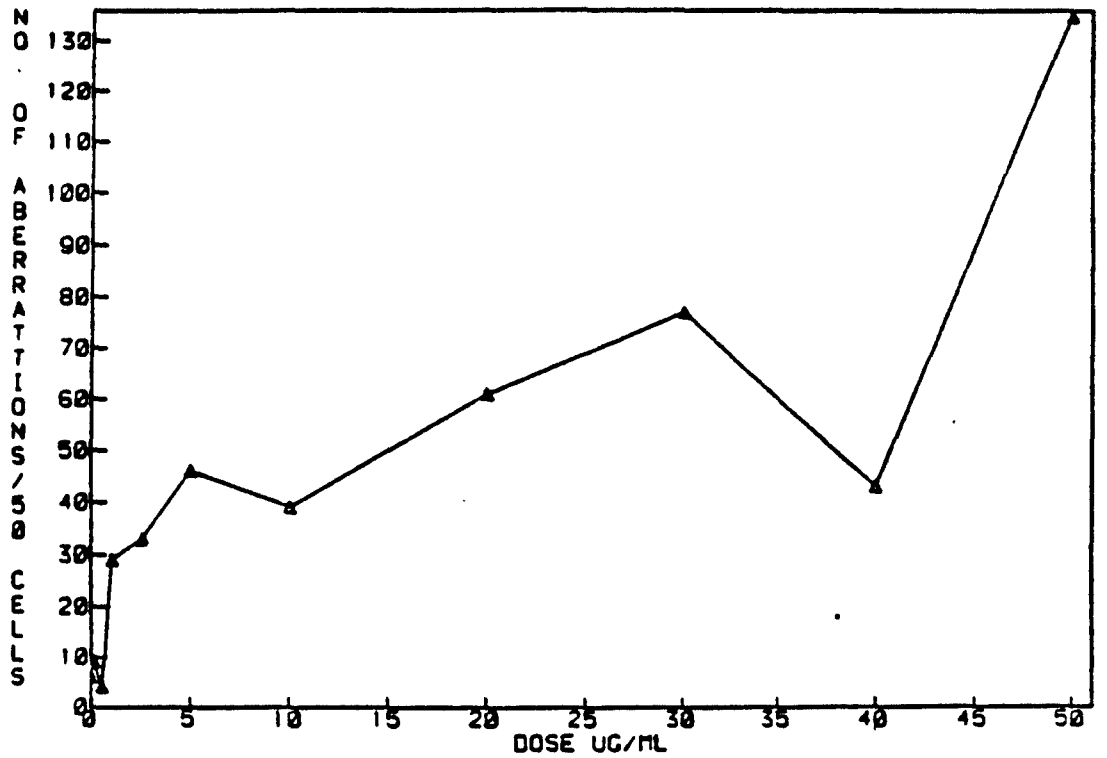

Figure 6: Gross aberration frequency in L5178Y/TK$^{+/-}$ Mouse Lymphoma cells following treatment with the >99.9% pure yellow dye, without exogenous metabolic activation.

TABLE 14. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE: SUMMARY/INDIVIDUAL ANIMAL

Treatment	Animal	SCE/Cell[a] Mean ± (S.D.)	Cell Kinetics (%)[b] M1	M2	M3
Negative Control	1	4 (1.7)	26.5	63.5	10.0
	2	5 (2.2)	20.0	71.5	8.5
	3	4 (1.8)	34.0	64.0	2.0
	4	No SCD[c]			
Solvent Control (DMSO + Corn Oil)	1	4 (2.0)	31.0	68.0	1.0
	2	3 (1.5)	28.0	66.0	6.0
	3	4 (1.7)	12.5	79.5	8.0
	4	4 (1.9)	12.0	82.0	6.0
Positive Control (Cyclophosphamide 15 mg/kg)	1	26 (6.8)	48.0	45.0	7.0
	2	33 (7.3)	50.0	47.0	3.0
	3	29 (8.1)	35.5	58.0	6.5
	4	29 (6.7)	57.0	42.0	1.0
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture					
10 mg/kg	1	4 (1.9)	20.5	74.5	5.0
	2	3 (1.4)	14.5	74.0	11.5
	3	4 (2.2)	11.6	78.4	10.0
	4	3 (2.0)	17.5	81.0	1.5
20 mg/kg	1	3 (1.5)	5.0	55.0	40.0
	2	4 (2.1)	13.5	64.5	22.0
	3	3 (1.5)	5.5	37.0	57.5
	4	No SCD			
40 mg/kg	1	3 (1.8)	12.0	29.5	58.5
	2	3 (1.6)	42.0	58.0	0.0
	3	3 (1.3)	23.5	60.9	15.6
	4	4 (2.0)	31.5	59.0	9.5

[a] - Mean of 30 cells/animal
[b] - Based on 200 spreads/animal
[c] - SCD= Sister Chromatid Differentiation

TABLE 15. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO DYE C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE: SUMMARY/TREATMENT GROUP

Treatment	Number of Animals	SCE/Cell[a] Mean ± (S.E.)	Cell Kinetics (%)[a] M1	M2	M3
Negative Control	3	4 (0.8)	27	66	7
Solvent Control (DMSO + Corn Oil)	4	4 (0.6)	21	74	5
Positive Control (Cyclophosphamide 15 mg/kg)	4	29 (2.6)	48	48	4
C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture					
10 mg/kg	4	3 (0.4)	16	77	7
20 mg/kg	3	3 (0.6)	8	52	40
40 mg/kg	4	3 (0.4)	27	52	21

[a] Mean of 3-4 animals/group

TABLE 16. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO C.I. SOLVENT YELLOW NO. 33: SUMMARY/INDIVIDUAL ANIMAL

Treatment	Animal	SCE/Cell[a] Mean ± (S.D.)	Cell Kinetics (%)[b] M1	M2	M3
Negative Control	1	4 (1.9)	33.5	54.0	12.5
	2	4 (2.1)	15.5	69.5	15.0
	3	4 (2.2)	17.5	77.5	5.0
	4	4 (1.9)	12.5	77.5	10.0
Solvent Control (DMSO)	1	4 (1.9)	6.0	83.0	11.0
	2	5 (2.1)	25.0	65.0	10.0
	3	5 (1.9)	12.5	80.0	7.5
	4	4 (1.3)	10.0	63.0	27.0
Positive Control (Cyclophosphamide 30 mg/kg)	1	57 (11.2)	79.0	21.0	0.0
	2	53 (9.6)	23.5	75.0	1.5
	3	50 (11.1)	29.0	65.0	6.0
	4	50 (10.6)	45.0	53.0	2.0
C.I. Solvent Yellow No 33					
5 mg/kg	1	5 (2.2)	14.0	70.0	16.0
	2	4 (2.1)	12.5	49.0	38.5
	3	5 (2.9)	8.0	60.0	32.0
	4	5 (2.6)	14.0	70.0	16.0
15 mg/kg	1	4 (1.9)	7.5	62.5	30.0
	2	5 (2.8)	6.0	70.0	24.0
	3	3 (1.1)	6.0	34.0	60.0
	4	No SCD[c]	-	-	-
25 mg/kg	1	2 (1.5)	14.5	46.5	39.0
	2	4 (2.3)	19.5	58.0	22.5
	3	3 (1.9)	13.5	26.5	60.0
	4	No SCD	-	-	-
35 mg/kg	1	2 (1.2)	2.5	14.5	83.0
	2	3 (1.6)	4.5	29.0	66.5
	3	4 (2.5)	14.0	56.5	29.5
	4	4 (2.6)	6.0	32.0	62.0

a Mean of 30 cells/animal
b Based on 200 spreads/animal
c SCD - Sister Chromatid Differentiation

TABLE 17. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO SINGLE EXPOSURE (I.P.) TO C.I. SOLVENT YELLOW NO. 33: SUMMARY/TREATMENT GROUP

Treatment	Number of Animals	SCE/Cell[a] Mean ± (S.E.)	Cell Kinetics (%)[a] M1	M2	M3
Negative Control	4	4 (0.3)	19.8	69.6	10.6
Solvent Control (DMSO)	4	5 (0.7)	13.4	72.8	13.8
Positive Control (Cyclophosphamide 30 mg/kg)	4	52 (3.5)	44.1	53.5	2.4
C.I. Solvent Yellow No. 33					
5 mg/kg	4	5 (0.5)	12.2	62.3	25.5
15 mg/kg	3	4 (0.8)	6.5	55.5	38.0
25 mg/kg	3	3 (0.7)	5.8	43.7	40.5
35 mg/kg	4	3 (0.7)	6.8	33.0	60.2

[a] mean of 3-4 animals/group

TABLE 18. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO REPEATED (OVER 3 DAYS) EXPOSURES (I.P.) TO C.I. SOLVENT YELLOW NO. 33: SUMMARY/INDIVIDUAL ANIMAL

Treatment	Animal	SCE/Cell[a] Mean ± (S.D.)		Cell Kinetics (%)[b] M1	M2	M3
Negative Control	1	3	(1.8)	12.0	73.0	15.0
	2	3	(1.9)	10.0	84.0	6.0
	3	3	(1.5)	5.0	85.0	10.0
	4	3	(1.2)	10.0	76.0	14.0
Solvent Control (DMSO)	1	4	(1.7)	4.0	66.0	30.0
	2	4	(2.6)	1.0	27.0	72.0
	3	4	(2.1)	4.0	64.0	32.0
	4	4	(1.6)	2.0	46.0	52.0
Positive Control (Cyclophosphamide 15 mg/kg)	1	33	(8.5)	6.0	82.0	12.0
	2	35	(8.7)	15.0	71.0	14.0
	3	33	(5.0)	4.0	84.0	12.0
	4	31	(7.3)	7.0	86.0	7.0
C.I. Solvent Yellow No. 33						
5 mg/kg/day	1	5	(2.0)	2.0	40.0	58.0
	2	4	(2.1)	0.0	49.0	51.0
	3	3	(1.2)	0.0	19.0	81.0
	4	4	(2.0)	0.0	32.0	68.0
15 mg/kg/day	1	4	(2.1)	4.0	59.0	37.0
	2	4	(1.9)	5.0	75.0	20.0
	3	3	(1.5)	4.0	57.0	39.0
	4	4	(1.7)	3.0	31.0	66.0
25 mg/kg/day	1	3	(1.5)	1.0	58.0	41.0
	2	4	(1.5)	2.0	36.0	62.0
	3	3	(1.9)	3.0	53.0	44.0
	4	3	(1.3)	0.0	13.0	87.0
35 mg/kg/day	1	3	(1.6)	0.0	6.0	94.0
	2	7	(2.5)	21.0	75.0	4.0
	3	4	(2.1)	0.0	54.0	46.0
	4	3	(1.4)	3.0	31.0	66.0

[a] Mean of 30 cells/animal
[b] Mean of 200 spreads/animal

TABLE 19. SCE AND CELL REPLICATION KINETICS ANALYSES OF MOUSE BONE MARROW CELLS AFTER IN VIVO REPEATED (OVER 3 DAYS) EXPOSURES (I.P.) TO C.I. SOLVENT YELLOW NO. 33: SUMMARY/TREATMENT GROUP

Treatment	Number of Animals	SCE/Cell[a] Mean ± (S.E.)		Cell Kinetics (%)[a] M1	M2	M3
Negative Control	4	3	(0.1)	9	80	11
Solvent Control (DMSO)	4	4	(0.2)	3	51	46
Positive Control (Cyclophosphamide 15 mg/kg)	4	33	(1.3)	8	81	11
C.I. Solvent Yellow No. 33						
5 mg/kg/day	4	4	(0.7)	1	35	64
15 mg/kg/day	4	4	(0.2)	4	55	41
25 mg/kg/day	4	3	(0.4)	1	40	59
35 mg/kg/day	4	4	(1.7)	6	42	52

[a] Mean of 4 animals/treatment

exceptional animal at the highest dose did reveal a small but significant SCE increase, and slowed cell-cycling as well. The exposure group as a whole did not reveal SCE induction or cytotoxicity effects.

Dye coloration or crystal evidence was not apparent in the peritoneum of animals dissected at the time of marrow cell harvest. There were also no crystals of dye evident in peritoneal cell pellets examined under a microscope. Peritoneal cell viabilities from 2 mice treated with 35 mg/kg C.I. Solvent Yellow No. 33 were similar to those of control (96.9% and 97.7% for treated animals vs. 95.2% and 97.5% for control animals). The percentage of cells represented as macrophages was also comparable between negative control and treated mice (83-84%). No traces of the dyes were observed in the marrow cell preparations.

Higher dose testing of both the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture and C.I. Solvent Yellow No. 33 was constrained by DMSO toxic effects and dye solubility in DMSO. Regardless of dye concentration, DMSO was determined in preliminary trials to inhibit cell cycling at injection volumes approximating 0.15 ml, and to cause animal death at higher doses. Doses considered to be at, or near, the limits of solubility in 0.1 ml of DMSO (20 mg/kg C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture; 15 mg/kg C.I. Solvent Yellow No. 33), and higher doses clearly as particulate suspensions, failed to give any clear evidence of SCE induction or cytotoxicity. Preliminary experiments suggesting cell-cycle delay effects from the dyes were not confirmed. It is now felt that these effects probably stemmed from the DMSO solvent initially used at higher concentrations. With the exception of one mouse revealing an approximate doubling of the control SCE values after multiple C.I. Solvent Yellow No. 33 injections at the highest dose, the data for both dyes were uniformly negative.

Although it is possible that the dyes may not have been distributed to the bone marrow, there was no evidence of dye localization in or around the peritoneal cavity. Further, there was no indication that peritoneal cells were stimulated by the dye. In the context of the present study, it is presumed that the I.P. injected dyes are distributed to marrow cells, and are inactive for SCE induction. Additional studies to evaluate effects after alternative routes of exposure (i.e. oral), in different cell-types (i.e. lung-from primary cell cultures established after inhalation exposure), and/or in cell cultures after in vitro exposure (so as to circumvent possible liver detoxification effects) are suggested if further confirmation of

negative activity for this genotoxic end-point is desired. In conclusion, cytogenetic evaluations, specifically SCE analysis in bone marrow cells of mice exposed in vivo, have not revealed any evidence of genotoxic potential associated with these dyes.

Conclusions

Two dyes, C.I. Solvent Yellow No. 33 and a C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 mixture were tested for mutagenicity in seven strains of Salmonella typhimurium, the L5178Y/$TK^{+/-}$ mouse lymphoma assay and in vivo in mice for sister chromatid exchange analysis. A purified C.I. Solvent Yellow No. 33 was tested in the seven strains of Salmonella typhimurium, and the L5178Y/$TK^{+/-}$ mouse lymphoma assay. In vivo, the two dyes were incapable of inducing SCEs. In vitro all three dyes gave a positive response for gene mutation in Salmonella strains TA102 and TA104. In vitro, all three dyes induced gene mutation at the TK locus in the mouse lymphoma assay. A large proportion of the mutants were small colonies predicting that the dyes might be clastogenic. (Preliminary studies for gross aberrations using mouse lymphoma cells confirm that the dyes can induce chromosome breaks, translocations and chromosome deletions.)

In evaluating the significance of the results from this test battery it is important to consider the differences between the in vivo and the in vitro results, as well as the different sensitivities of the endpoints. The negative in vivo results could have resulted from the non-genotoxic nature of the dyes, the failure of the dyes to reach the target tissue, or the specific inability of the dyes to induce SCEs. In performing the tests, care was taken to observe that dye crystals were not apparent in the peritoneum of animals dissected at the time of marrow cell harvest.

The ability of the dyes to induce SCEs was also questioned. In preliminary studies (Doerr and Moore, unpublished data, U.S. EPA, 1984) the pure yellow dye was evaluated for its ability to induce SCEs in mouse lymphoma cells in vitro. The results were negative. It appears therefore that the negative in vivo results may be due to the insensitivity of the endpoint to the dyes rather than a true in vivo non-genotoxicity of the dyes. If a further evaluation of these dyes is desired, it would be interesting to test them in vivo for the induction of gross aberrations and in a different cell line (possibly human lymphocytes) in vitro for gross aberrations.

The purified C.I. Solvent Yellow No. 33 was tested to determine if the dye itself or the impurities were responsible for the observed mutagenic activity. This purified dye was found to be mutagenic in Salmonella, and at the TK locus of mouse lymphoma cells. It was also found to be clastogenic to mouse lymphoma cells. From these studies it is clear that the dye itself, not an impurity, is mutagenic. This dye is present both in the yellow dye and the green-yellow mixture.

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Appendix A

Data and statistical analysis for the Salmonella typhimurium bioassays of 3 Army dyes. The data is ordered in the following manner. Data sheets for individual experiments which include testing of a single compound both with and without activation are followed by two pages showing the statistical analysis of the data with and without activation. Cultures with S-9 activation are indicated as RLA026 or RLA027. BMGS-84-0001 is the code for the C.I. Solvent Green No. 3 - C.I. Solvent Yellow No. 33 Mixture; BMGS-84-0002 is C.I. Solvent Yellow No. 33; and BMGS-84-0003 is the purified yellow dye.

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: GBBA ON 03/30/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1CC

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1179	1205	1160			1188.00	14.73
2-AA	RLA026	0.50	363	360	298			340.33	36.69
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	105	101	103			103.00	2.00
	-	100.000	103	134	97			111.33	19.86
BMGS-84-0001									
	RLA026	1.00	108	103				105.50	3.54
	RLA026	5.00	126	132				129.00	4.24
	RLA026	10.00	142	109				125.50	23.33
	RLA026	30.00	133	104				118.50	20.51
	RLA026	50.00	138	106				122.00	22.63
	RLA026	100.00	173	165				169.00	5.66
	RLA026	300.00	153	144				148.50	6.36
	RLA026	500.00	161	158				159.50	2.12
	RLA026	1000.00	142	134				138.00	5.66
	-	1.00	114	128				121.00	9.90
	-	5.00	102	144				123.00	29.70
	-	10.00	108	72				90.00	25.46
	-	30.00	132	127				129.50	3.54
	-	50.00	116	125				120.50	6.36
	-	100.00	131	113				122.00	12.73
	-	300.00	110	138				124.00	19.80
	-	500.00	165	145				155.00	14.14
	-	1000.00	131	117				124.00	9.90

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GB&A ON 03/30/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA100

*RLA626

ABOVE 100 UG/PLATE, THE SAMPLE APPEARS TO PRECIPITATE OUT OF SOLUTION.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	105	101	103	103.00	2.00
.50*	UGS	363	360	298	340.33	36.69
1.00	UGS	108	103		105.50	3.54
5.00	UGS	126	132		129.00	4.24
10.00	UGS	142	109		125.50	23.33
30.00	UGS	133	104		116.50	20.51
50.00	UGS	138	106		122.00	22.63
100.00	UGS	173	165		169.00	5.66
300.00	UGS	153	144		148.50	6.36

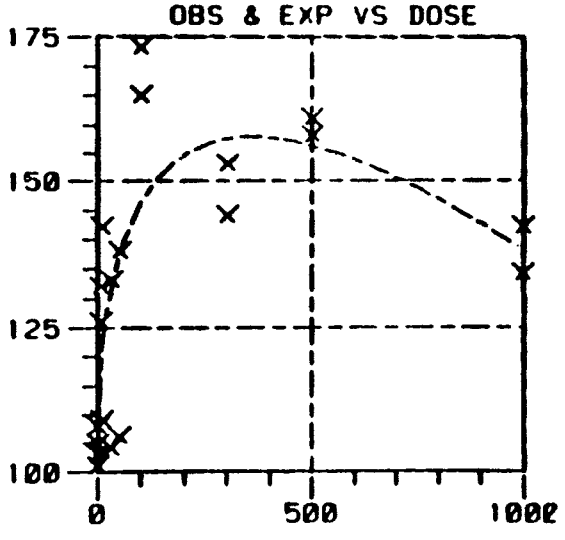
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	103.565	1.8926	.4446	.00058

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	13 14 11		.2842	-76.8837
ADEQUACY	17.22	6	.0085	-85.4925
TOXICITY	9.36	1	.0022	-90.1714
MUTAGENICITY	54.67	2	.0000	-112.8263

AVERAGE SLOPE (NONLIN. MODEL) = .210
95% CONF. LIMITS = (.096, .461)

AVERAGE SLOPE (LINEAR REGR.) = .095
95% CONF. LIMITS = (.040, .150)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	103	134	97	111.33	19.86
3.00*	UGS	1179	1205	1180	1188.00	14.73
1.00	UGS	114	128		121.00	9.90
5.00	UGS	102	144		123.00	29.70
10.00	UGS	108	72		90.00	25.46
30.00	UGS	132	127		129.50	3.54
50.00	UGS	116	125		120.50	6.36
100.00	UGS	131	113		122.00	12.73
300.00	UGS	110	138		124.00	19.80

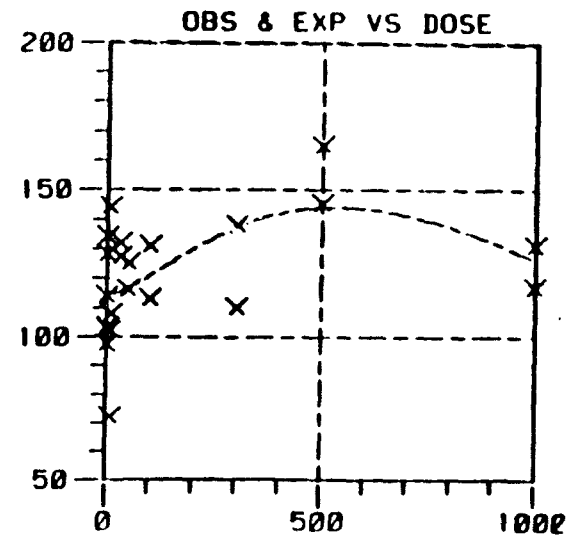
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	114.862	-2.4707	1.2399	.00149

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	29.27	11	.0021	-84.1549
ADEQUACY	21.64	6	.0014	-94.9766
TOXICITY	5.91	1	.0151	-97.9301
MUTAGENICITY	17.72	2	.0001	-103.8347

AVERAGE SLOPE (NONLIN. MODEL) = .375
95% CONF. LIMITS = (.133, 1.059)

AVERAGE SLOPE (LINEAR REGR.) = .071
95% CONF. LIMITS = (.020, .121)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA100

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1253	1350	1320			1307.67	49.66
2-NF	RLA026	0.50	953	814	842			869.00	74.28
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	115	125	114			118.00	6.08
	-	100.000	109	102	110			107.00	4.36
BMGS-84-0001									
	RLA026	1.00	129	127				128.00	1.41
	RLA026	5.00	143	141				142.00	1.41
	RLA026	10.00	158	173				165.50	10.61
	RLA026	30.00	146	154				150.00	5.66
	RLA026	50.00	151	164				157.50	9.19
	RLA026	100.00	185	166				176.50	13.02
	RLA026	300.00	173	153				163.00	14.14
	-	1.00	113	129				121.00	11.31
	-	5.00	125	161				143.00	25.46
	-	10.00	115	114				114.50	0.71
	-	30.00	130	131				130.50	0.71
	-	50.00	137	117				127.00	14.14
	-	100.00	109	111				110.00	1.41
	-	300.00	129	128				128.50	0.71

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

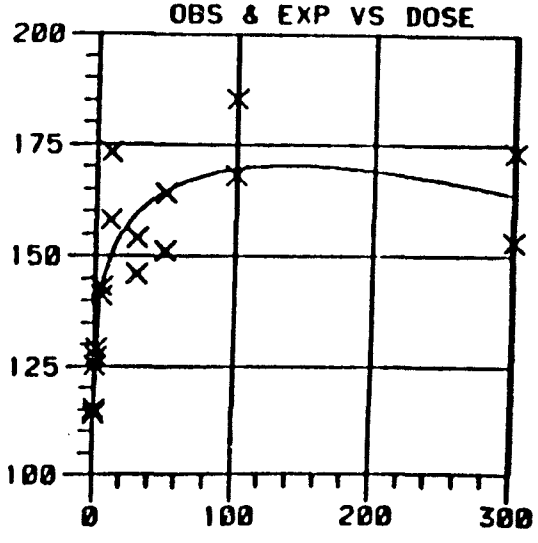
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	115	125	114	118.00	6.08
.50*	UGS	953	812	842	869.00	74.28
1.00	UGS	129	127		128.00	1.41
5.00	UGS	143	141		142.00	1.41
10.00	UGS	158	173		165.50	10.61
30.00	UGS	146	154		150.00	5.66
50.00	UGS	151	164		157.50	9.19
100.00	UGS	185	168		176.50	12.02
300.00	UGS	173	153		163.00	14.14

	B(0)	B(1)	B(2)	B(3)
ESTS.	117.484	2.7833	.3095	.00086

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.13	9	.9025	-60.0989
ADEQUACY	6.33	4	.1761	-63.2616
TOXICITY	2.67	1	.1024	-64.5952
MUTAGENICITY	38.20	2	.0000	-82.3596

AVERAGE SLOPE (NONLIN. MODEL) = .673
95% CONF. LIMITS = (.324, 1.396)

AVERAGE SLOPE (LINEAR REGR.) = .460
95% CONF. LIMITS = (.231, .688)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	109	102	110	107.00	4.36
3.00*	UGS	1253	1350	1320	1307.67	49.66
1.00	UGS	113	129		121.00	11.31
5.00	UGS	125	161		143.00	25.46
10.00	UGS	115	114		114.50	.71
30.00	UGS	130	131		130.50	.71
50.00	UGS	137	117		127.00	14.14
100.00	UGS	109	111		110.00	1.41
300.00	UGS	129	128		128.50	.71

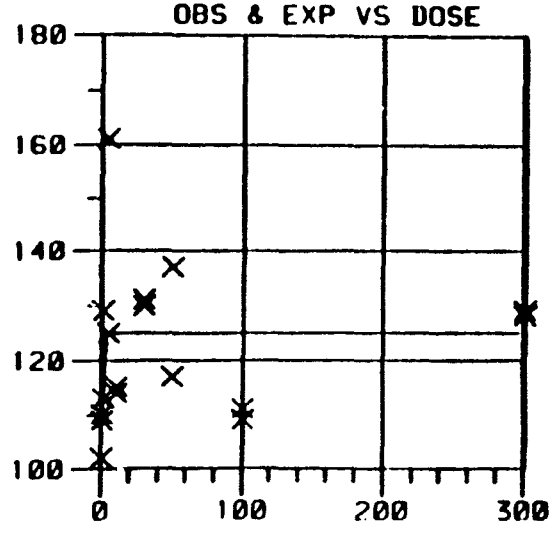
	B(0)	B(1)	B(2)
ESTS.	107.000	2.8846	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	7.55	9	.5801	-60.1832
ADEQUACY	11.51	5	.0421	-65.9395
MUTAGENICITY	6.74	2	.0343	-69.3111

AVERAGE SLOPE (NONLIN. MODEL) = .060
95% CONF. LIMITS = (-.008, .473)

AVERAGE SLOPE (LINEAR REGR.) = .019
95% CONF. LIMITS = (-.057, .094)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: PLATE TEST - PREINCUBATION STRAIN: TA100

COMPOUND	ACT	UGS PER PLATE	MISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1227	1278	1267			1257.33	26.84
2-AA	RLA026	0.50	354	307	337			332.67	23.80
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	108	117	115			113.33	4.73
	-	100.000	144	132	127			134.33	8.74
BMGS-84-0001									
	RLA026	1.00	115	137				126.00	15.56
	RLA026	5.00	137	141				139.00	2.83
	RLA026	10.00	105	149				127.00	31.11
	RLA026	30.00	139	138				138.50	0.71
	RLA026	50.00	165	170				167.50	3.54
	RLA026	100.00	166	184				175.00	12.73
	RLA026	300.00	163	152				157.50	7.78
	-	1.00	105	142				123.50	26.16
	-	5.00	108	131				119.50	16.26
	-	10.00	137	115				126.00	15.56
	-	30.00	145	145				145.00	0.00
	-	50.00	161	129				145.00	22.63
	-	100.00	117	120				118.50	2.12
	-	300.00	117	118				117.50	0.71

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

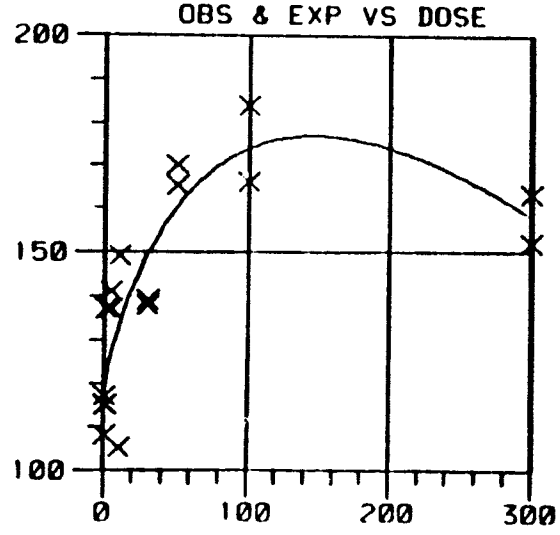
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	108	117	115	113.33	4.73
.50*	UGS	354	307	337	332.67	23.80
1.00	UGS	115	137		126.00	15.56
5.00	UGS	137	141		139.00	2.83
10.00	UGS	105	149		127.00	31.11
30.00	UGS	139	138		138.50	.71
50.00	UGS	165	170		167.50	3.54
100.00	UGS	166	184		175.00	12.73
300.00	UGS	163	152		157.50	7.78

	B(0)	B(1)	B(2)	B(3)
ESTS.	118.077	1.1529	.7883	.00311

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	11.38	9	.2504	-63.3098
ADEQUACY	6.06	4	.1949	-66.3387
TOXICITY	6.57	1	.0104	-69.6224
MUTAGENICITY	46.50	2	.0000	-89.5874

AVERAGE SLOPE (NONLIN. MODEL) = 1.195
95% CONF. LIMITS = (.471, 3.029)

AVERAGE SLOPE (LINEAR REGR.) = .579
95% CONF. LIMITS = (.359, .798)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

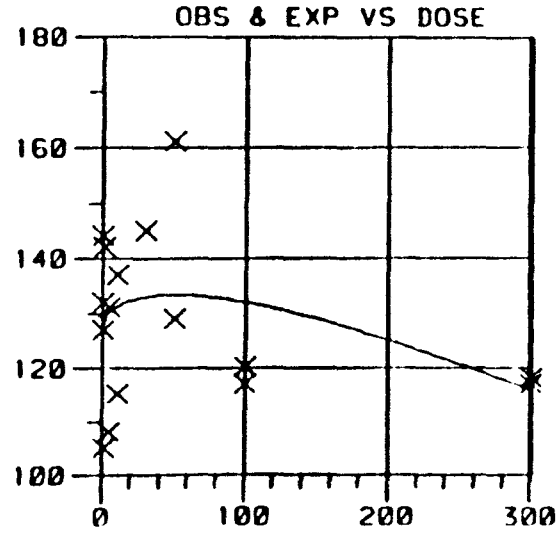
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	144	132	127	134.33	8.74
3.00*	UGS	1227	1278	1267	1257.33	26.84
1.00	UGS	105	142		123.50	26.16
5.00	UGS	108	131		119.50	16.26
10.00	UGS	137	115		126.00	15.56
30.00	UGS	145	145		145.00	.00
50.00	UGS	161	129		145.00	22.63
100.00	UGS	117	120		118.50	2.12
300.00	UGS	117	118		117.50	.71

	B(0)	B(1)	B(2)	B(3)
ESTS.	127.988	.0841	.6859	.00151

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	14.39	9	.1092	-64.0920
ADEQUACY	10.61	4	.0313	-69.3993
TOXICITY	3.59	1	.0582	-71.1938
MUTAGENICITY	1.27	2	.5292	-70.0357

AVERAGE SLOPE (NONLIN. MODEL) = .374
95% CONF. LIMITS = (.008, 18.518)

AVERAGE SLOPE (LINEAR REGR.) = .541
95% CONF. LIMITS = (-.252, 1.333)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLA027	30.00	1253	1203	1096			1184.00	80.21
	-	0.50	1451	1509	1507			1489.00	32.92
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	269	325	302			298.67	28.15
	-	100.000	215	213	203			210.33	6.43
BMGS-84-0001									
	RLA027	10.00	311	336				324.50	19.09
	RLA027	30.00	395	448				421.50	37.48
	RLA027	50.00	480	526				503.00	32.53
	RLA027	100.00	586	631				608.50	31.82
	RLA027	300.00	654	694				674.00	28.28
	-	10.00	301	290				295.50	7.78
	-	30.00	380	413				396.50	23.33
	-	50.00	456	407				431.50	34.65
	-	100.00	477	389				433.00	62.23
	-	300.00	500	383				441.50	82.73

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

*RLA627

POSITIVE CONTROL USED WAS DANTHRON.

-

MITOMYCIN-C WAS USED AS THE POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/05/84 TECHNICIAN: MJK

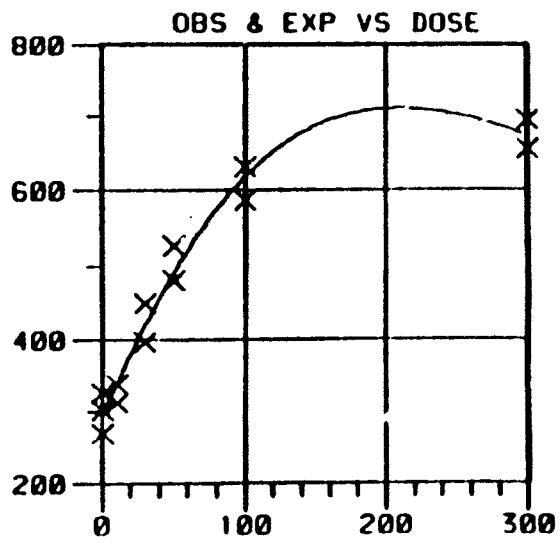
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	269	325	302	298.67	28.15
30.00*	UGS	1253	1203	1096	1184.00	80.21
10.00	UGS	311	338		324.50	19.09
30.00	UGS	395	448		421.50	37.48
50.00	UGS	480	526		503.00	32.53
100.00	UGS	586	631		608.50	31.82
300.00	UGS	654	694		674.00	28.28

	B(0)	B(1)	B(2)	B(3)
ESTS.	295.096	1.5877	1.0599	.00418

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	14.72	7	.0398	-58.8369
ADEQUACY	2.06	2	.3572	-59.8663
TOXICITY	37.55	1	.0000	-78.6427
MUTAGENICITY	570.44	2	.0000	-345.0842

AVERAGE SLOPE (NONLIN. MODEL) = 6.883
95% CONF. LIMITS = (4.256, 11.130)

AVERAGE SLOPE (LINEAR REGR.) = 1.194
95% CONF. LIMITS = (.746, 1.642)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/05/84 TECHNICIAN: MJA

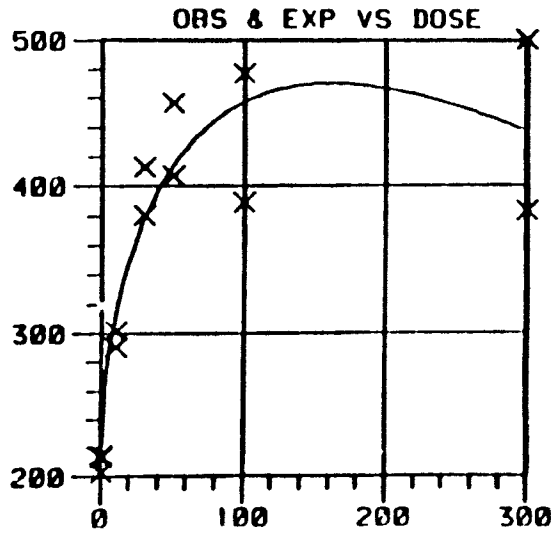
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	215	213	203	210.33	6.43
.50*	UGS	1451	1509	1507	1489.00	32.92
10.00	UGS	301	290		295.50	7.78
30.00	UGS	380	413		396.50	23.33
50.00	UGS	456	407		431.50	34.65
100.00	UGS	477	389		433.00	62.23
300.00	UGS	500	383		441.50	82.73

	B(0)	B(1)	B(2)	B(3)
ESTS.	208.958	3.5406	.5085	.00216

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	29.20	7	.0001	-64.4863
ADEQUACY	8.06	2	.0178	-68.5167
TOXICITY	19.97	1	.0000	-78.5011
MUTAGENICITY	340.27	2	.0000	-238.6500

AVERAGE SLOPE (NONLIN. MODEL) = 5.041
95% CONF. LIMITS = (4.279, 5.940)

AVERAGE SLOPE (LINEAR REGR.) = 4.497
95% CONF. LIMITS = (3.414, 5.580)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLAC27	30.00		(4)1176	1208			1192.00	22.63
	-	0.50	1182	1166	1170			1173.33	7.57
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	257	234	239			243.33	12.10
	-	100.000	154		(4) 145			149.50	0.36
BMGS-84-0001									
	RLA027	10.00		(4)	270			270.00	0.00
	RLA027	30.00	412		400			406.00	8.49
	RLA027	50.00	453		437			445.00	11.31
	RLA027	100.00	547		507			527.00	28.28
	RLA027	300.00	684		660			672.00	16.97
	-	10.00	214		203			208.50	7.78
	-	30.00	375		330			352.50	31.82
	-	50.00	346		392			369.00	32.53
	-	100.00	432		449			440.50	12.02
	-	300.00	482		490			486.00	5.66

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 5000GS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAY: WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1G2

BACKGROUNDS:

(4)CONTAMINATED

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1C2

*RLAC27

DANTHRON WAS USED AS A POSITIVE CONTROL.

MITOMYCIN C WAS USED AS A POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 – C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/08/84 TECHNICIAN: MJK

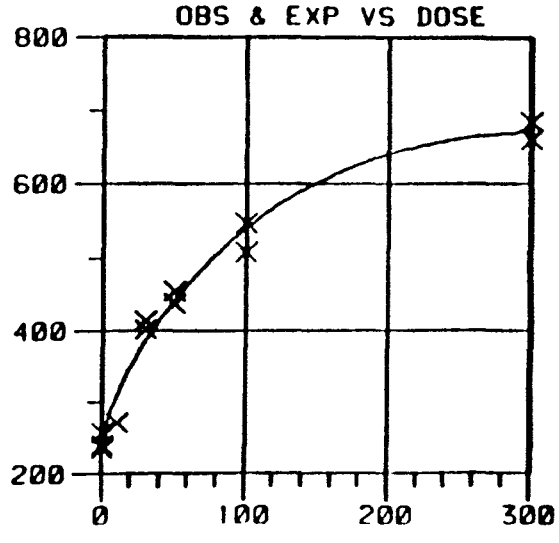
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	257	234	239	243.33	12.10
30.00*	UGS		1176	1208	1192.00	22.63
10.00	UGS		270		270.00	.00
30.00	UGS	412	400		406.00	8.49
50.00	UGS	453	437		445.00	11.31
100.00	UGS	547	507		527.00	28.28
300.00	UGS	684	660		672.00	16.97

	B(0)	B(1)	B(2)	B(3)
ESTS.	240.569	2.5598	.7509	.00188

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.61	6	.7287	-48.7529
ADEQUACY	7.92	2	.0191	-52.7105
TOXICITY	7.93	1	.0049	-56.6762
MUTAGENICITY	646.84	2	.0000	-376.1290

AVERAGE SLOPE (NONLIN. MODEL) = 3.123
95% CONF. LIMITS = (1.896, 5.145)

AVERAGE SLOPE (LINEAR REGR.) = 1.307
95% CONF. LIMITS = (.914, 1.699)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/08/84 TECHNICIAN: MJK

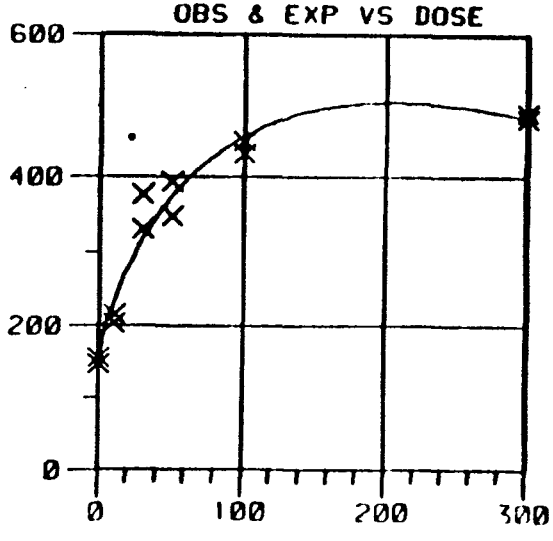
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	154	145		149.50	6.36
.50*	UGS	1182	1168	1170	1173.33	7.57
10.00	UGS	214	203		208.50	7.78
30.00	UGS	375	330		352.50	31.82
50.00	UGS	346	392		369.00	32.53
100.00	UGS	432	449		440.50	12.02
300.00	UGS	482	490		486.00	5.66

	B(0)	B(1)	B(2)	B(3)
ESTS.	146.049	2.9088	.7012	.00288

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	6.69	6	.3500	-48.7717
ADEQUACY	14.02	2	.0009	-55.7794
TOXICITY	31.93	1	.0000	-71.7453
MUTAGENICITY	569.27	2	.0000	-340.4157

AVERAGE SLOPE (NONLIN. MODEL) = 3.335
95% CONF. LIMITS = (2.056, 5.409)

AVERAGE SLOPE (LINEAR REGR.) = .900
95% CONF. LIMITS = (.427, 1.373)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/15/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLA027	0.50	1252	1377	1392	1370		1347.75	64.49
	-	30.00	1380	1320	1383			1361.00	35.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	264	264	266			264.67	1.15
	-	100.000	202	186	213			201.00	12.53
EMGS-84-0001									
	RLA027	1.00	281	268				274.50	9.19
	RLA027	3.00	286	252				269.00	24.04
	RLA027	10.00	293	281				287.00	8.49
	RLA027	30.00	338	383				360.50	31.82
	RLA027	50.00	426	380				403.00	32.53
	RLA027	100.00	601	530				565.50	50.20
	RLA027	300.00	712	716				714.00	2.83
	-	1.00	203	190				196.50	9.19
	-	3.00	216	255				235.50	27.58
	-	10.00	241	236				239.50	2.12
	-	30.00	328	312				320.00	11.31
	-	50.00	348	428				388.00	56.57
	-	100.00	437	449				443.00	8.49
	-	300.00	457	436				446.50	14.85

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 5.0UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA CN 06/15/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

*RLAG27

MITOMYCIN C WAS USED AS THE POSITIVE CONTROL.

DANTHRON WAS USED AS THE POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

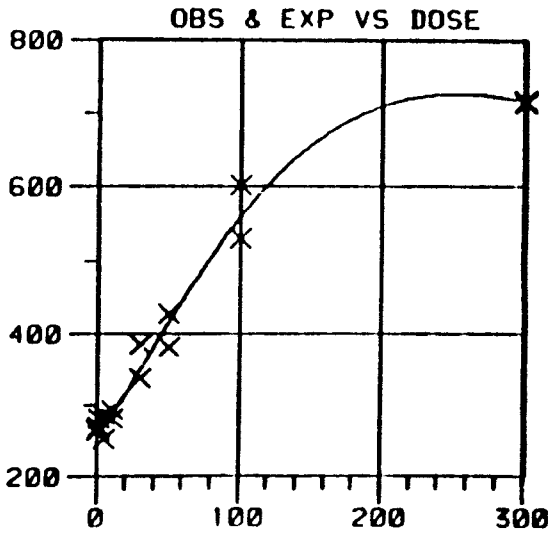
DOSE	UNITS	PLATE COUNTS				MEAN	S.D.
.00	UGS	264	264	266		264.67	1.16
.50*	UGS	1252	1377	1392	1370	1347.75	64.49
1.00	UGS	281	268			274.50	9.19
5.00	UGS	286	252			269.00	24.04
10.00	UGS	293	281			287.00	8.49
30.00	UGS	338	383			360.50	31.82
50.00	UGS	426	380			403.00	32.53
100.00	UGS	601	530			565.50	50.20
300.00	UGS	712	716			714.00	2.83

	B(0)	B(1)	B(2)	B(3)
ESTS.	267.118	.5843	1.2620	.00439

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	12.62	9	.1806	-71.9629
ADEQUACY	2.26	4	.6889	-73.0906
TOXICITY	26.94	1	.0000	-86.5600
MUTAGENICITY	957.75	2	.0000	-551.9666

AVERAGE SLOPE (NONLIN. MODEL) = 7.993
95% CONF. LIMITS = (2.377, 26.875)

AVERAGE SLOPE (LINEAR REGR.) = 1.547
95% CONF. LIMITS = (1.260, 1.833)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

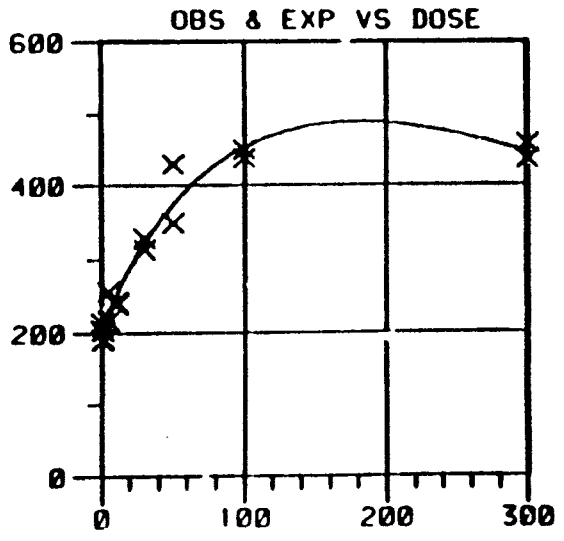
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	202	188	213	201.00	12.53
30.00*	UGS	1380	1320	1383	1361.00	35.54
1.00	UGS	203	190		196.50	9.19
5.00	UGS	216	255		235.50	27.58
10.00	UGS	241	238		239.50	2.12
30.00	UGS	328	312		320.00	11.31
50.00	UGS	348	428		388.00	56.57
100.00	UGS	437	449		443.00	8.49
300.00	UGS	457	436		446.50	14.85

	B(0)	B(1)	B(2)	B(3)
ESTS.	197.093	2.1277	.8743	.00388

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	14.54	9	.1042	-70.9985
ADEQUACY	3.96	4	.4108	-72.9809
TOXICITY	50.55	1	.0000	-98.2539
MUTAGENICITY	552.01	2	.0000	-348.9849

AVERAGE SLOPE (NONLIN. MODEL) = 5.133
95% CONF. LIMITS = (4.358, 6.046)

AVERAGE SLOPE (LINEAR REGR.) = 3.767
95% CONF. LIMITS = (3.101, 4.433)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1C4

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-AA	RLAC27	3.00	2424	2366	2444			2405.33	50.65
OTHER PUS	-	50.00	1736	1657	1786			1726.33	65.04
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	350	316	290			318.67	30.09
	-	100.000	241	222	252			238.33	15.18
BMGS-84-0001									
	RLAC27	1.00	316	354				334.00	26.28
	RLAC27	5.00	316	354				334.00	26.28
	RLAC27	10.00	361	360				360.50	0.71
	RLAC27	30.00	450	455				452.50	3.54
	RLAC27	50.00	532	505				518.50	19.09
	RLAC27	100.00	544	516				531.00	18.38
	RLAC27	300.00	512	396				454.00	82.02
	-	1.00	300	242				271.00	41.01
	-	5.00	263	205				234.00	41.01
	-	10.00	266	262				264.00	2.83
	-	30.00	283	287				285.00	2.83
	-	50.00	328	280				304.00	33.94
	-	100.00	330	325				327.50	3.54
	-	300.00	305	289				297.00	11.31

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 5.000GS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GB8A ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

METHYL GLYOXAL WAS USED AS THE POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN: MJK

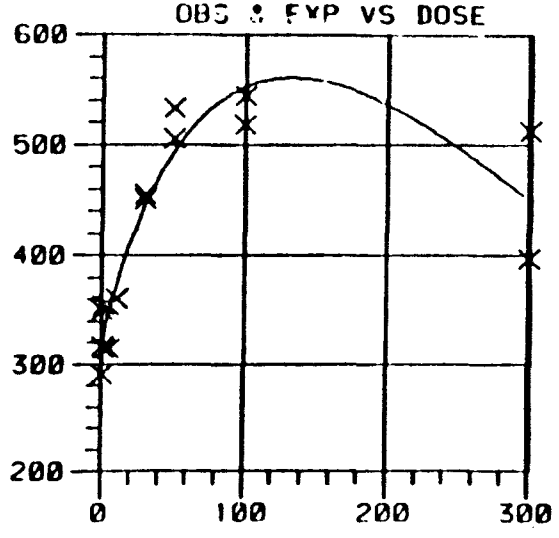
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	350	316	290	318.67	30.09
3.00*	UGS	2424	2348	2444	2405.33	50.65
1.00	UGS	314	354		334.00	28.28
5.00	UGS	314	354		334.00	28.28
10.00	UGS	361	360		360.50	.71
30.00	UGS	450	455		452.50	3.54
50.00	UGS	532	505		518.50	19.09
100.00	UGS	544	518		531.00	18.38
300.00	UGS	512	396		454.00	82.02

	B(0)	B(1)	B(2)	B(3)
ESTS.	315.927	2.3098	.8702	.00454

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	26.66	9	.0016	-79.8677
ADEQUACY	7.30	4	.1208	-83.5187
TOXICITY	78.24	1	.0000	-122.6363
MUTAGENICITY	270.32	2	.0000	-218.6802

AVERAGE SLOPE (NONLIN. MODEL) = 6.062
95% CONF. LIMITS = (5.258, 6.988)

AVERAGE SLOPE (LINEAR REGR.) = 4.041
95% CONF. LIMITS = (3.430, 4.651)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN: MJK

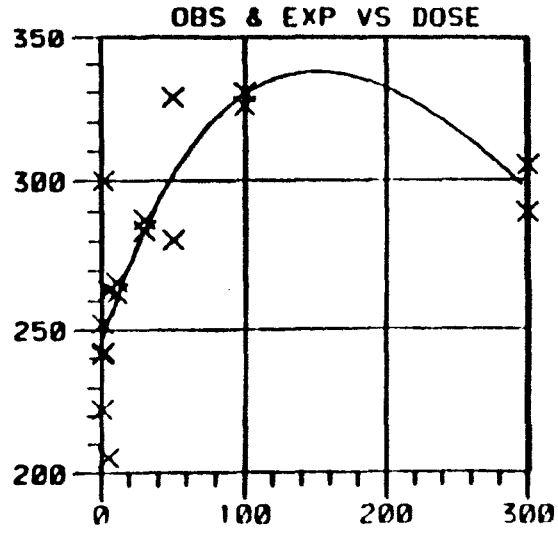
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	241	222	252	238.33	15.18
50.00*	UGS	1736	1657	1786	1726.33	65.04
1.00	UGS	300	242		271.00	41.01
5.00	UGS	263	205		234.00	41.01
10.00	UGS	266	262		264.00	2.83
30.00	UGS	283	287		285.00	2.83
50.00	UGS	328	280		304.00	33.94
100.00	UGS	330	325		327.50	3.54
300.00	UGS	305	289		297.00	11.31

	B(0)	B(1)	B(2)	B(3)
ESTS.	245.663	.9130	.9951	.00396

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	19.64	9	.0202	-73.1519
ADEQUACY	8.24	4	.0833	-77.2695
TOXICITY	11.81	1	.0006	-83.1734
MUTAGENICITY	50.29	2	.0000	-102.4142

AVERAGE SLOPE (NONLIN. MODEL) = 2.444
95% CONF. LIMITS = (1.176, 5.080)

AVERAGE SLOPE (LINEAR REGR.) = 1.183
95% CONF. LIMITS = (.404, 1.963)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-AA	RLA027	5.00	2053	2002	2086			2047.00	42.32
OTHER POS	-	50.00	1810	1716	1780			1769.33	46.92
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	363	402	375			380.00	19.97
	-	100.00U	277	266	279			274.00	7.00
BMGS-84-0001									
	RLA027	1.00	353	351				352.00	1.41
	RLA027	5.00	383	381				382.00	1.41
	RLA027	10.00	405	370				387.50	24.75
	RLA027	30.00	416	469				442.50	37.48
	RLA027	50.00	505	466				485.50	27.58
	RLA027	100.00	532	532				532.00	0.00
	RLA027	300.00	532	524				528.00	5.66
	-	1.00	298	250				274.00	33.94
	-	5.00	290	268				279.00	15.56
	-	10.00	305	318				311.50	9.19
	-	30.00	317	319				318.00	1.41
	-	50.00	308	317				312.50	6.36
	-	100.00	376	376				376.00	0.00
	-	300.00	330	352				341.00	15.56

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

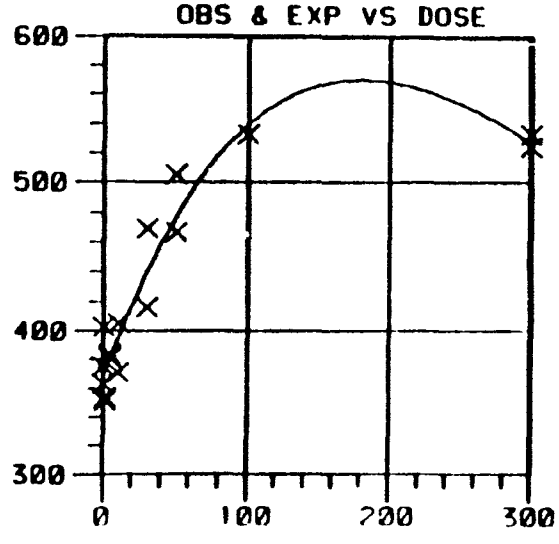
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	363	402	375	380.00	19.97
3.00*	UGS	2053	2002	2086	2047.00	42.32
1.00	UGS	353	351		352.00	1.41
5.00	UGS	383	381		382.00	1.41
10.00	UGS	405	370		387.50	24.75
30.00	UGS	416	469		442.50	37.48
50.00	UGS	505	466		485.50	27.58
100.00	UGS	532	532		532.00	.00
300.00	UGS	532	524		528.00	5.66

	B(0)	B(1)	B(2)	B(3)
ESTS	367.656	1.4110	1.0030	.00374

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.49	9	.4854	-71.3638
ADEQUACY	3.81	4	.4317	-73.2709
TOXICITY	22.50	1	.0000	-84.5231
MUTAGENICITY	166.13	2	.0000	-156.3335

AVERAGE SLOPE (NONLIN. MODEL) = 4.157
95% CONF. LIMITS = (1.543, 11.203)

AVERAGE SLOPE (LINEAR REGR.) = 1.729
95% CONF. LIMITS = (1.369, 2.089)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

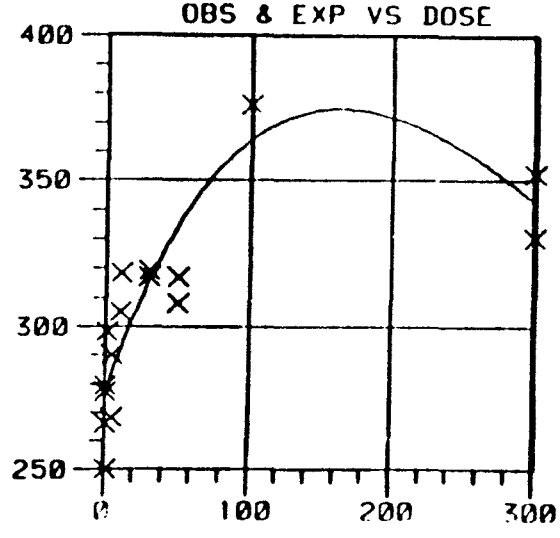
DOSE	UNITS	PLATE COUNTS			MEAN	S.E
.00	UGS	277	266	279	274.00	7.00
50.00*	UGS	1810	1718	1780	1769.33	46.92
1.00	UGS	298	250		274.00	33.94
5.00	UGS	290	268		279.00	15.56
10.00	UGS	305	318		311.50	9.19
30.00	UGS	317	319		318.00	1.41
50.00	UGS	308	317		312.50	6.36
100.00	UGS	376	376		376.00	.00
300.00	UGS	330	52		341.00	15.56

	B(0)	B(1)	B(2)	B(3)
ESTS.	275.931	1.0077	9704	.00347

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.55	9	.6842	-67.5713
ADEQUACY	6.88	4	.1426	-71.0095
TOXICITY	9.43	1	.0021	-75.7233
MUTAGENICITY	54.17	2	.0000	-98.0952

AVERAGE SLOPE (NONLIN. MODEL) = 2.391
95% CONF. LIMITS = (.859, 6.653)

AVERAGE SLOPE (LINEAR REGR.) = .942
95% CONF. LIMITS = (.689, 1.194)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1535

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1068	1057	1018			1047.67	26.27
2-AA	RLA027	3.00	154	154	157			155.00	1.73
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	32	33	18			27.67	6.39
	-	100.000	55	50	40			48.33	7.64
BMGS-84-0001									
	RLA027	10.00	25	15				20.50	6.36
	RLA027	30.00	24	29				26.50	3.54
	RLA027	50.00	28	29				28.50	0.71
	RLA027	100.00	17	20				18.50	2.12
	RLA027	300.00	14	21				17.50	4.95
	-	10.00	61	52				56.50	6.36
	-	30.00	28	32				30.00	2.83
	-	50.00	40	32				36.00	5.66
	-	100.00	51	41				46.00	7.07
	-	300.00	36	42				39.00	4.24

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: MJK

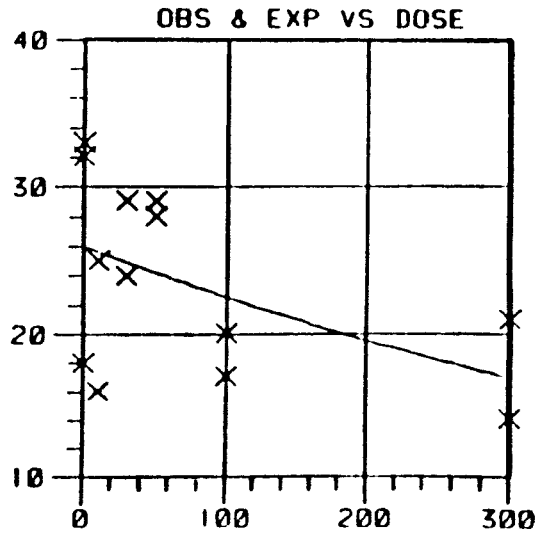
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	32	33	18	27.67	8.39
3.00*	UGS	154	154	157	155.00	1.73
10.00	UGS	25	16		20.50	6.36
30.00	UGS	24	29		26.50	3.54
50.00	UGS	28	29		28.50	.71
100.00	UGS	17	20		18.50	2.12
300.00	UGS	14	21		17.00	4.95

	B(0)	B(1)	B(2)	B(3)
ESTS.	25.973*********		.0000	.00144

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	9.19	7	.2391	-37.0998
ADEQUACY	5.74	2	.0568	-39.9689
TOXICITY	5.54	1	.0185	-42.7413
MUTAGENICITY	.00	21	.0000	-39.9689

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = .050
95% CONF. LIMITS = (-.162, .262)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	55	50	40	48.33	7.64
3.00*	UGS	1068	1057	1018	1047.67	26.27
10.00	UGS	61	52		56.50	6.36
30.00	UGS	28	32		30.00	2.83
50.00	UGS	40	32		36.00	5.66
100.00	UGS	51	41		46.00	7.07
300.00	UGS	36	42		39.00	4.24

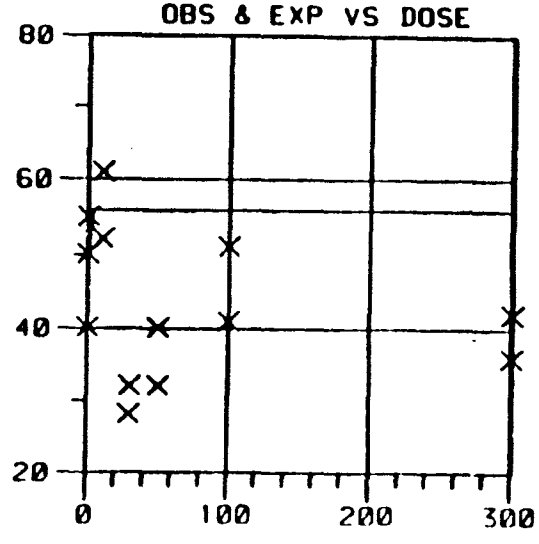
	B(0)	B(1)	B(2)
ESTS.	48.333	2.0168	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	5.83	7	.5592	-39.2126
ADEQUACY	60.07	3	.0000	-69.2456
MUTAGENICITY	.01	2	.9950	-50.2144

AVERAGE SLOPE (NONLIN. MODEL) = .075
95% CONF. LIMITS = (.022, .258)

AVERAGE SLOPE (LINEAR REGR.) = -.066
95% CONF. LIMITS = (-.253, .120)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBGA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1535

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1003	979	1014			998.67	17.90
2-AA	RLAC27	3.00	89	103	109			100.33	10.26
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	25	24	18			22.33	3.79
	-	100.000	43	26	28			32.33	9.29
BMGS-84-0001									
	RLAC27	10.00	14	14				14.00	0.00
	RLAC27	30.00	19	15				17.00	2.83
	RLAC27	50.00	17	18				17.50	0.71
	RLAC27	100.00	21	21				21.00	0.00
	RLAC27	300.00	11	9				10.00	1.41
	-	10.00	36	27				31.50	6.36
	-	30.00	36	17				26.50	13.44
	-	50.00	24	33				28.50	6.36
	-	100.00	41	27				34.00	9.90
	-	300.00	21	17				19.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/08/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	25	24	18	22.33	3.79
3.00*	UGS	89	103	109	100.33	10.26
10.00	UGS	14	14		14.00	.00
30.00	UGS	19	15		17.00	2.83
50.00	UGS	17	18		17.50	.71
100.00	UGS	21	21		21.00	.00
300.00	UGS	11	9		10.00	1.41

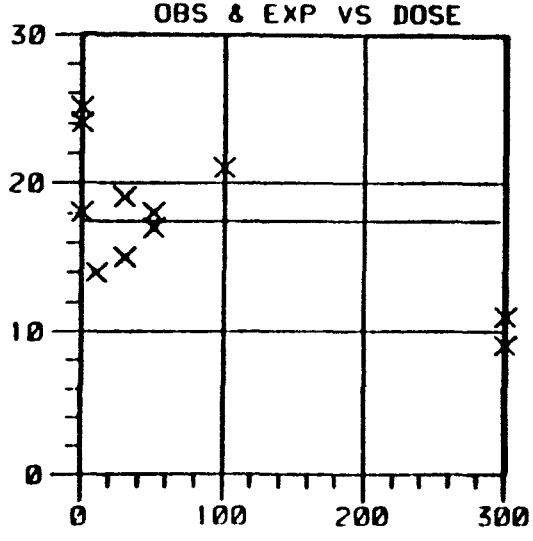
	B(0)	B(1)	B(2)
ESTS.	17.386	-663.7156	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	1.98	7	.9608	-31.3414
ADEQUACY	14.13	3	.0027	-38.4079
MUTAGENICITY	.00	21	.0000	-38.4079

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = .010
95% CONF. LIMITS = (-.057, .076)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/08/84 TECHNICIAN: MJK

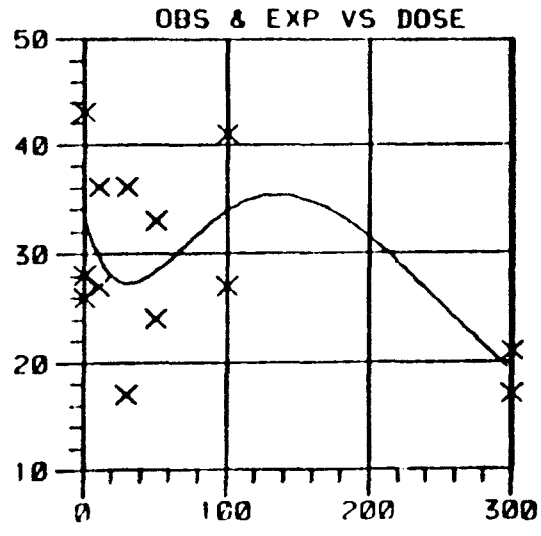
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	43	26	28	32.33	9.29
3.00*	UGS	1003	979	1014	998.67	17.00
10.00	UGS	36	27		31.50	6.36
30.00	UGS	36	17		26.50	13.44
50.00	UGS	24	33		28.50	6.36
100.00	UGS	41	27		34.00	9.90
300.00	UGS	21	17		19.00	2.83

	B(0)	B(1)	B(2)	B(3)
ESTS.	33.140	-5.1746	2.1060	.01309

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	18.16	7	.0113	-42.6575
ADEQUACY	.32	2	.8511	-42.8187
TOXICITY	14.73	1	.0001	-50.1831
MUTAGENICITY	3.94	2	.1393	-44.7902

AVERAGE SLOPE (NONLIN. MODEL) = .428
95% CONF. LIMITS = (.203, .904)

AVERAGE SLOPE (LINEAR REGR.) = -.097
95% CONF. LIMITS = (-.373, .179)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1537

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
9-AA	-	100.00	1258	1335	1452			1348.33	97.68
2-AA	RLA027	3.00	561	472	408			480.33	76.84
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	20	16	17			17.67	2.08
	-	100.000	9	8	9			8.33	0.58
BMGS-84-0031									
	RLA027	10.00	14	28				21.00	9.90
	RLA027	30.00	25	20				22.50	3.54
	RLA027	50.00	39	21				30.00	12.73
	RLA027	100.00	30	24				27.00	4.24
	RLA027	300.00	27	26				26.50	0.71
	-	10.00	16	12				14.00	2.83
	-	30.00	14	17				15.50	2.12
	-	50.00	14	21				17.00	4.24
	-	100.00	16	14				15.00	1.41
	-	300.00	16	13				14.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50.0UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: MJK

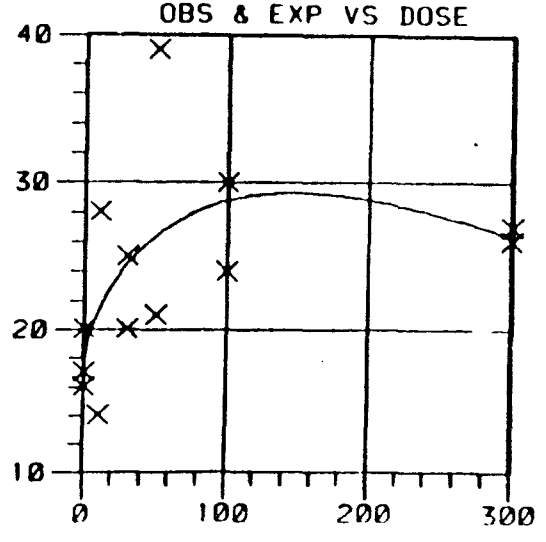
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	20	16	17	17.67	2.08
3.00*	UGS	561	472	408	480.33	76.84
10.00	UGS	14	28		21.00	9.90
30.00	UGS	25	20		22.50	3.54
50.00	UGS	39	21		30.00	12.73
100.00	UGS	30	24		27.00	4.24
300.00	UGS	27	26		26.50	.71

	B(0)	B(1)	B(2)	B(3)
ESTS.	17.567	-.0943	.6743	.00276

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	11.80	7	.1074	-38.2940
ADEQUACY	1.50	2	.4723	-39.0441
TOXICITY	1.25	1	.2627	-39.6713
MUTAGENICITY	9.33	2	.0094	-43.7073

AVERAGE SLOPE (NONLIN. MODEL) = .255
95% CONF. LIMITS = (.034, 1.924)

AVERAGE SLOPE (LINEAR REGR.) = .226
95% CONF. LIMITS = (.004, .448)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	8	8	9	8.33	.58
100.00*	UGS	1258	1335	1452	1348.33	97.69
10.00	UGS	16	12		14.00	2.83
30.00	UGS	14	17		15.50	2.12
50.00	UGS	14	20		17.00	4.24
100.00	UGS	16	14		15.00	1.41
300.00	UGS	16	13		14.50	2.12

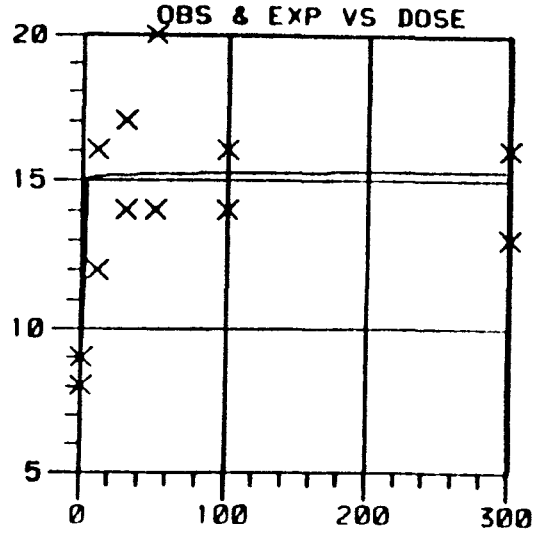
	B(0)	B(1)	B(2)
ESTS.	8.333	1.8945	.0081

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	2.44	7	.9312	-29.9930
ADEQUACY	.68	3	.8768	-30.3354
MUTAGENICITY	8.92	2	.0115	-34.7979

AVERAGE SLOPE (NONLIN. MODEL) = .137
95% CONF. LIMITS = (.007, 2.882)

AVERAGE SLOPE (LINEAR REGR.) = .162
95% CONF. LIMITS = (.067, .257)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1537

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
9-AA	-	100.00	512	605	453			523.33	76.63
2-AA	RLA027	3.00	314	316	311			313.67	2.52
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	20	16	16			18.00	2.00
	-	100.000	12	7	17			12.00	5.00
BMGS-84-0001									
	RLA027	10.00	26	15				20.50	7.78
	RLA027	30.00	20	31				25.50	7.78
	RLA027	50.00	21	24				22.50	2.12
	RLA027	100.00	(4)	39				39.00	0.00
	RLA027	300.00	24	29				26.50	3.54
	-	10.00	25	21				23.00	2.83
	-	30.00	17	16				17.50	0.71
	-	50.00	13	16				14.50	2.12
	-	100.00	18	16				17.00	1.41
	-	300.00	24	27				25.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SCLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN

RESEARCH LAB: GBBA ON 06/C8/84 C8/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1537

BACKGROUNDS:

(4)CONTAMINATED

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: MJK

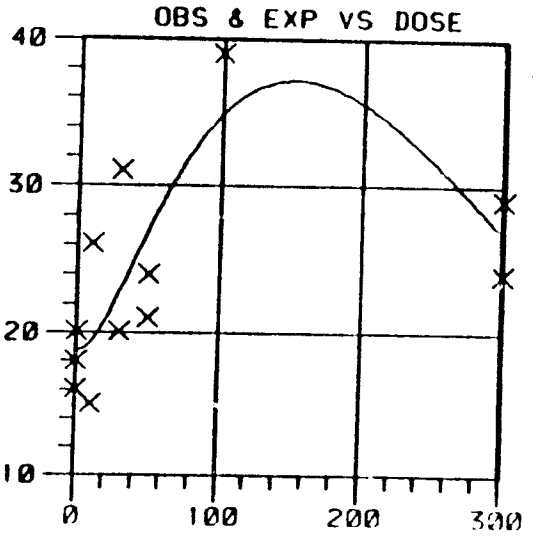
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	20	18	16	18.00	2.00
3.00*	UGS	314	316	311	313.67	2.52
10.00	UGS	26	15		20.50	7.78
30.00	UGS	20	31		25.50	7.78
50.00	UGS	21	24		22.50	2.12
100.00	UGS		39		39.00	.00
300.00	UGS	24	29		26.50	3.54

	B(0)	B(1)	B(2)	B(3)
ESTS.	18.817	-2.6207	1.4612	.00829

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.44	6	.3758	-33.0643
ADEQUACY	2.97	2	.2262	-34.5505
TOXICITY	3.84	1	.0501	-36.4701
MUTAGENICITY	12.07	2	.0024	-40.5855

AVERAGE SLOPE (NONLIN. MODEL) = .609
95% CONF. LIMITS = (.098, 3.761)

AVERAGE SLOPE (LINEAR REGR.) = .180
95% CONF. LIMITS = (.078, .283)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	12	7	17	12.00	5.00
100.00*	UGS	512	605	453	523.33	76.63
10.00	UGS	25	21		23.00	2.83
30.00	UGS	17	18		17.50	.71
50.00	UGS	13	16		14.50	2.12
100.00	UGS	18	16		17.00	1.41
300.00	UGS	24	27		25.50	2.12

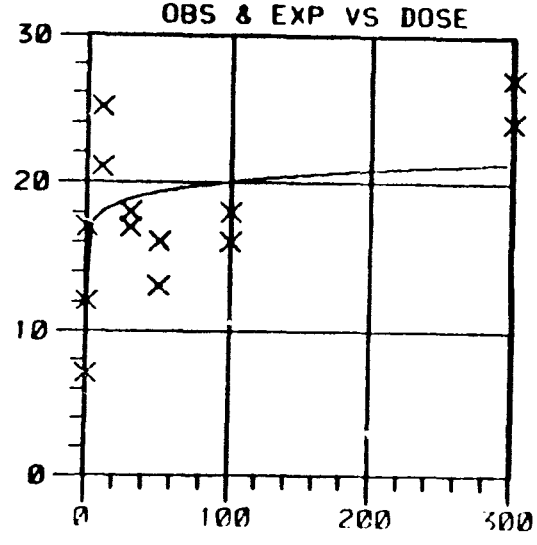
	B(0)	B(1)	B(2)
ESTS.	12.093	1.4476	.1353

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	5.15	7	.6420	-33.0167
ADEQUACY	8.01	3	.0458	-37.0219
MUTAGENICITY	8.44	2	.0147	-41.2401

AVERAGE SLOPE (NONLIN. MODEL) = .031
95% CONF. LIMITS = (015, .064)

AVERAGE SLOPE (LINEAR REGR.) = .032
95% CONF. LIMITS = (.007, .057)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	615	652	628			631.67	18.77
2-AA	RLA027	0.50	1061	1063	1052			1058.67	5.86
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	41	19	36			32.00	11.53
	-	100.000	18	13	15			15.33	2.52
BMGS-84-0001									
	RLA027	10.00	36	33				34.50	2.12
	RLA027	30.00	42	33				37.50	6.36
	RLA027	50.00	32	40				36.00	5.66
	RLA027	100.00	40	26				33.00	9.90
	RLA027	300.00	19	19				19.00	0.00
	-	10.00	19	16				17.50	2.12
	-	30.00	17	17				17.00	0.00
	-	50.00	20	15				17.50	3.54
	-	100.00	100	19				59.50	57.28
	-	300.00	15	12				13.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/05/84 TECHNICIAN: MJK

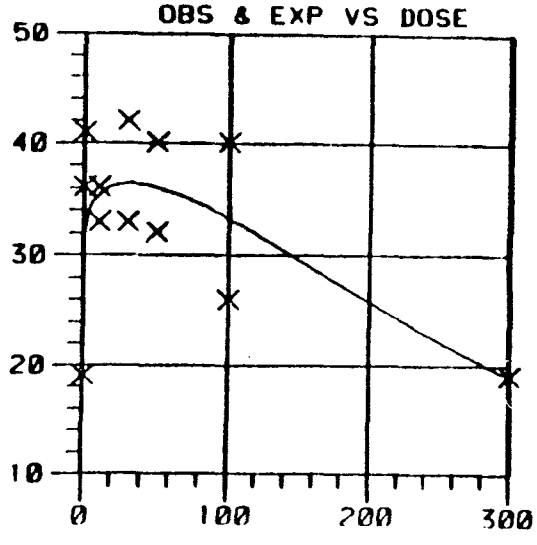
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	41	19	36	32.00	11.53
.50*	UGS	1061	1063	1052	1058.67	5.86
10.00	UGS	36	33		34.50	2.12
30.00	UGS	42	33		37.50	6.36
50.00	UGS	32	40		36.00	5.66
100.00	UGS	40	26		33.00	9.90
300.00	UGS	19	19		19.00	.00

	B(0)	B(1)	B(2)	B(3)
ESTS.	31.963	.2290	.5908	.00429

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	13.38	7	.0633	-41.3010
ADEQUACY	.11	2	.9443	-41.3584
TOXICITY	19.40	1	.0000	-51.0603
MUTAGENICITY	3.46	2	.1774	-43.0876

AVERAGE SLOPE (NONLIN. MODEL) = .254
95% CONF. LIMITS = (.000, ********)

AVERAGE SLOPE (LINEAR REGR.) = .088
95% CONF. LIMITS = (-.157, .333)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: CBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/05/84 TECHNICIAN: MJK

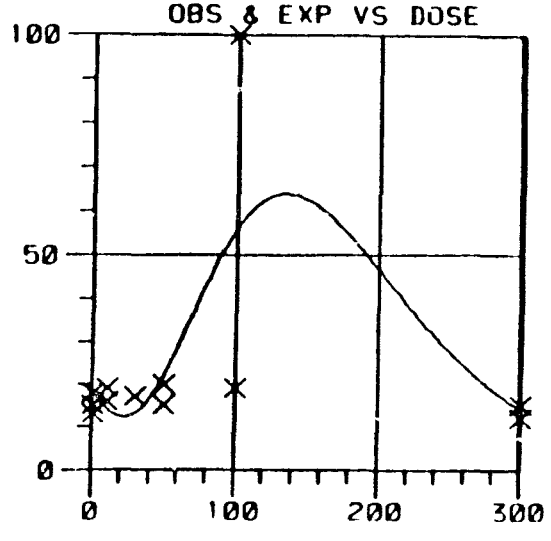
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	18	13	15	15.33	2.52
3.00*	UGS	615	652	628	631.67	18.77
10.00	UGS	19	16		17.50	2.12
30.00	UGS	17	17		17.00	.00
50.00	UGS	20	15		17.50	3.54
100.00	UGS	100	19		59.50	57.28
300.00	UGS	15	12		13.50	2.12

	B(0)	B(1)	B(2)	B(3)
ESTS	18.419	-9.7494	3.5567	.02628

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	57.27	7	.0000	-62.3009
ADEQUACY	7.73	2	.0210	-66.1655
TOXICITY	86.01	1	.0000	-109.1693
MUTAGENICITY	110.96	2	.0000	-121.6466

AVERAGE SLOPE (NONLIN. MODEL) = 7.574

AVERAGE SLOPE (LINEAR REGR.) = .413
95% CONF. LIMITS = (.057, .769)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	502	604	528			544.67	53.00
2-AA	RLA027	3.50	816	807	828			817.00	10.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	60	62	73			71.67	9.07
	-	100.000	21	16	19			19.33	1.53
BMGS-34-3001									
	RLA027	10.00	52	50				51.00	1.41
	RLA027	30.00	80	67				73.50	9.19
	RLA027	50.00	57	62				59.50	3.54
	RLA027	100.00	47	65				56.00	12.73
	RLA027	300.00	38	23				30.50	10.61
	-	10.00	15	12				13.50	2.12
	-	30.00	16	18				17.00	1.41
	-	50.00	17	12				14.50	3.54
	-	100.00	13	15				14.00	1.41
	-	300.00	11	16				13.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 5000GS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 -
C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: G88A ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

*RLA027

SPONTANEOUS COUNT IS HIGH DUE TO SMALL SALMONELLA COLONIES ON THE PLATE.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/08/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	80	62	73	71.67	9.07
.50*	UGS	816	807	828	817.00	10.54
10.00	UGS	52	50		51.00	1.41
30.00	UGS	80	67		73.50	9.19
50.00	UGS	57	62		59.50	3.54
100.00	UGS	47	65		56.00	12.73
300.00	UGS	38	23		30.50	10.61

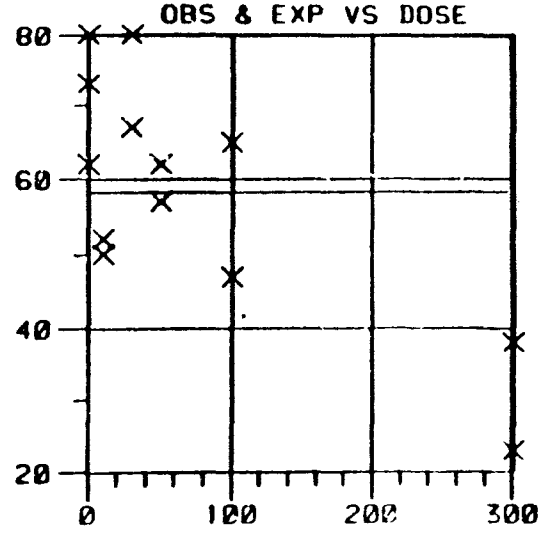
	B(0)	B(1)	B(2)
ESTS.	58.174	**********	

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	10.28	7	.1734	-43.2544
ADEQUACY	50.17	3	.0000	-68.3413
MUTAGENICITY	.00	2	.9999	-68.3414

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = -.082
95% CONF. LIMITS = (-.486, .322)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/08/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	21	18	19	19.33	1.53
3.00*	UGS	502	604	528	544.67	53.00
10.00	UGS	15	12		13.50	2.12
30.00	UGS	16	18		17.00	1.41
50.00	UGS	17	12		14.50	3.54
100.00	UGS	13	15		14.00	1.41
300.00	UGS	11	16		13.50	3.54

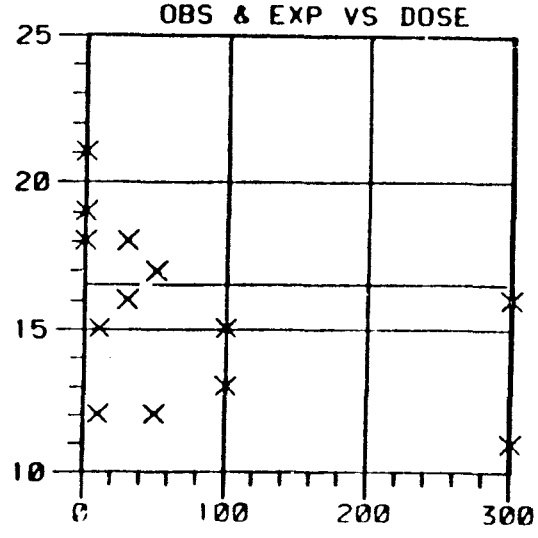
	B(0)	B(1)	B(2)
ESTS.	16.515	**********	659.4364

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	2.62	7	.9175	-31.0826
ADEQUACY	5.44	3	.1421	-33.8042
MUTAGENICITY	.01	2	.9950	-33.2910

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = -.065
95% CONF. LIMITS = (-.165, .036)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 06/20/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	492	504	551			515.67	31.18
2-AA	RLA027	0.50	668	714	737			706.33	35.13
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	45	39	36			40.00	4.58
	-	100.000	8	12	16			12.00	4.00
BMGS-84-0001									
	RLA027	10.00	41	31				36.00	7.07
	RLA027	30.00	50	41				45.50	6.36
	RLA027	50.00	38	39				38.50	0.71
	RLA027	100.00	51	48				49.50	2.12
	RLA027	300.00	25	27				26.00	1.41
	-	10.00	9	15				12.00	4.24
	-	30.00	13	16				14.50	2.12
	-	50.00	17	18				17.50	0.71
	-	100.00	19	17				18.00	1.41
	-	300.00	21	16				18.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

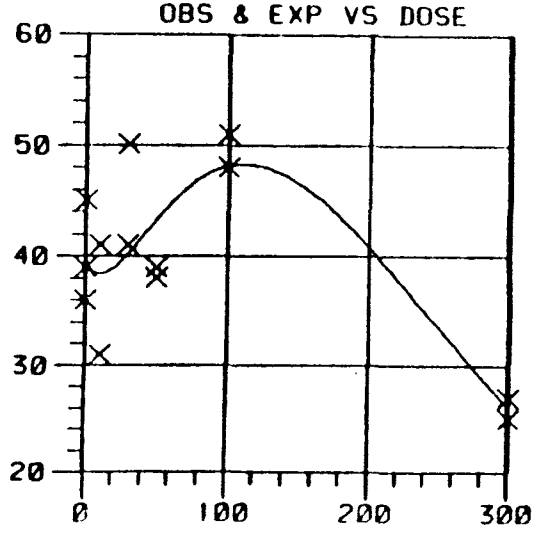
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	45	39	36	40.00	4.58
.50*	UGS	668	714	737	706.33	35.13
10.00	UGS	41	31		36.00	7.07
30.00	UGS	50	41		45.50	6.36
50.00	UGS	38	39		38.50	.71
100.00	UGS	51	48		49.50	2.12
300.00	UGS	25	27		26.00	1.41

	B(0)	B(1)	B(2)	B(3)
ESTS.	39.614	-2.4808	1.5179	.00998

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.51	7	.8342	-37.4507
ADEQUACY	2.84	2	.2416	-38.8712
TOXICITY	15.96	1	.0001	-46.8525
MUTAGENICITY	8.13	2	.0172	-42.9354

AVERAGE SLOPE (NONLIN. MODEL) = .909
95% CONF. LIMITS = (.063, 13.066)

AVERAGE SLOPE (LINEAR REGR.) = .097
95% CONF. LIMITS = (.007, .187)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

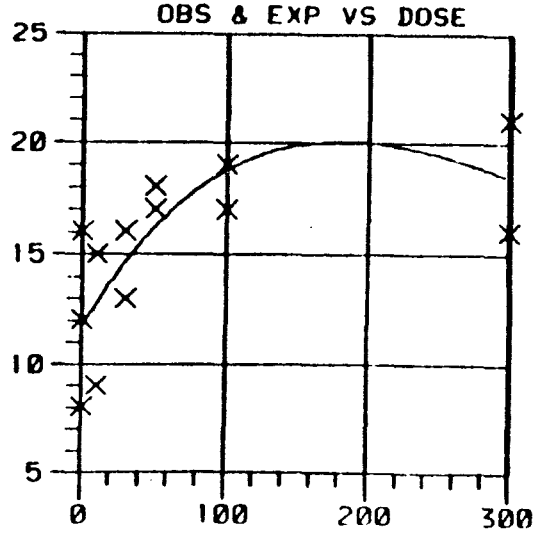
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	8	12	16	12.00	4.00
3.00*	UGS	492	504	551	515.67	31.18
10.00	UGS	9	15		12.00	4.24
30.00	UGS	13	16		14.50	2.12
50.00	UGS	17	18		17.50	.71
100.00	UGS	19	17		18.00	1.41
300.00	UGS	21	16		18.50	3.54

	B(0)	B(1)	B(2)	B(3)
ESTS.	11.743	-1.8610	1.0086	.00398

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	5.29	7	.6243	-32.1536
ADEQUACY	.39	2	.8241	-32.3471
TOXICITY	.86	1	.3539	-32.7768
MUTAGENICITY	6.51	2	.0386	-35.6013

AVERAGE SLOPE (NONLIN. MODEL) = .161
95% CONF. LIMITS = (.005, 5.347)

AVERAGE SLOPE (LINEAR REGR.) = .112
95% CONF. LIMITS = (.014, .210)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBdA ON 03/30/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA98

COMPOUND	ACT	uGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	300	312	315			309.00	7.94
2-AA	RLA026	0.50	875	837	853			855.00	19.08
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	60	41	48			49.67	9.61
	-	100.000	28	33	30			30.33	2.52
BMGS-84-0001									
	RLA026	1.00	51	50				50.50	0.71
	RLA026	5.00	50	40				45.00	7.07
	RLA026	10.00	53	42				47.50	7.78
	RLA026	30.00	53	48				50.50	3.54
	RLA026	50.00	49	58				53.50	6.36
	RLA026	100.00	54	57				55.50	2.12
	RLA026	300.00	49	61				55.00	8.49
	RLA026	500.00	63	47				55.00	11.31
	RLA026	1000.00	52	26				39.00	18.38
	-	1.00	24	27				25.50	2.12
	-	5.00	27	25				26.00	1.41
	-	10.00	32	54				43.00	15.56
	-	30.00	26	30				28.00	2.83
	-	50.00	25	25				25.00	0.00
	-	100.00	29	20				24.50	6.36
	-	300.00	19	26				22.50	4.95
	-	500.00	21	25				23.00	2.83
	-	1000.00	25	18				21.50	4.95

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY 5-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-PM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	60	41	48	49.67	9.61
.50*	UGS	875	837	853	855.00	19.08
1.00	UGS	51	50		50.50	.71
5.00	UGS	50	40		45.00	7.07
10.00	UGS	53	42		47.50	7.78
30.00	UGS	53	48		50.50	3.54
50.00	UGS	49	58		53.50	6.36
100.00	UGS	54	57		55.50	2.12
300.00	UGS	49	61		55.00	8.49

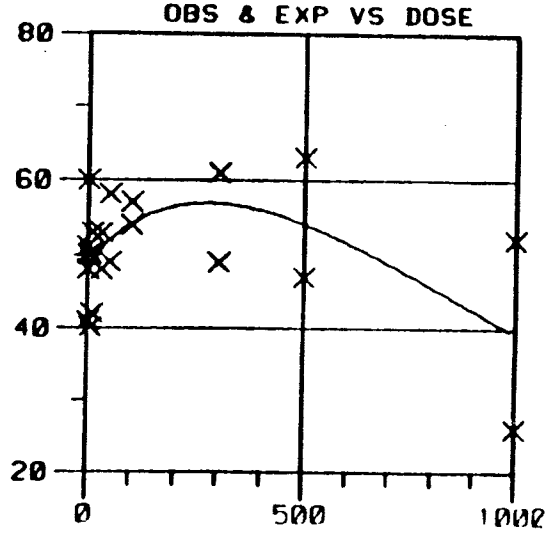
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	48.329	-1.6163	.9319	.00148

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	19.50	11	.0527	-70.0896
ADEQUACY	1.36	6	.9684	-70.7680
TOXICITY	8.76	1	.0031	-75.1486
MUTAGENICITY	7.00	2	.0301	-74.2698

AVERAGE SLOPE (NONLIN. MODEL) = .170
95% CONF. LIMITS = (.007, 3.888)

AVERAGE SLOPE (LINEAR REGR.) = -.330
95% CONF. LIMITS = (-1.479, .818)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	28	33	30	30.33	2.52
3.00*	UGS	300	312	315	309.00	7.94
1.00	UGS	24	27		25.50	2.12
5.00	UGS	27	25		26.00	1.41
10.00	UGS	32	54		43.00	15.56
30.00	UGS	26	30		28.00	2.83
50.00	UGS	25	25		25.00	.00
100.00	UGS	29	20		24.50	6.36
300.00	UGS	19	26		22.50	4.95

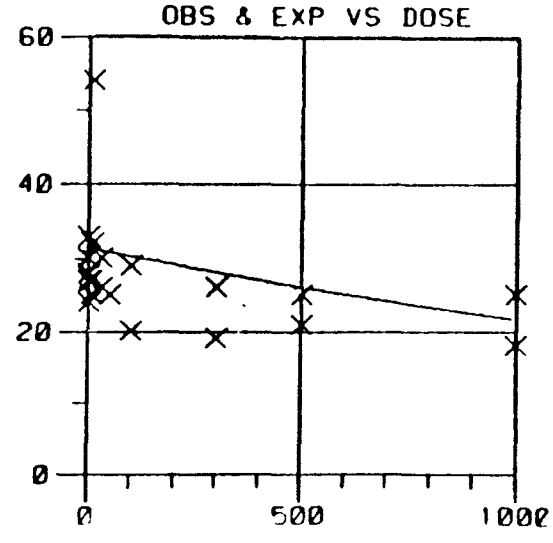
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	30.469	- 0631	.0000	.00038

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	10.81	11	.4590	-59.1673
ADEQUACY	20.57	6	.0022	-69.4521
TOXICITY	15.89	1	.0001	-77.3966
MUTAGENICITY	.01	2	.9950	-67.5145

AVERAGE SLOPE (NONLIN. MODEL) = .094
95% CONF. LIMITS = (.000,********)

AVERAGE SLOPE (LINEAR REGR.) = 1.269
95% CONF. LIMITS = (-.077, 2.615)



MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA98

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	250	270	255			258.33	10.41
2-AA	RLA026	0.50	740	825	817			794.00	46.94
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	42	31	43			38.67	6.66
	-	100.000	23	24	26			24.00	4.58
BMGS-84-0001									
	RLA026	1.00	37	40				38.50	2.12
	RLA026	5.00	61	45				53.00	11.31
	RLA026	10.00	29	32				30.50	2.12
	RLA026	30.00	56	48				52.00	5.66
	RLA026	50.00	45	53				49.00	5.66
	RLA026	100.00	50	48				49.00	1.41
	RLA026	300.00	50	56				53.00	4.24
	-	1.00	26	27				26.50	0.71
	-	5.00	21	29				25.00	5.66
	-	10.00	25	31				28.00	4.24
	-	30.00	26	27				26.50	0.71
	-	50.00	30	29				29.50	0.71
	-	100.00	41	18				29.50	16.26
	-	300.00	20	31				25.50	7.78

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE GREEN
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA98

*RLA02a

ABOVE 100 UG/PLATE, THE SAMPLE APPEARS TO PRECIPITATE OUT OF SOLUTION.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: -
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

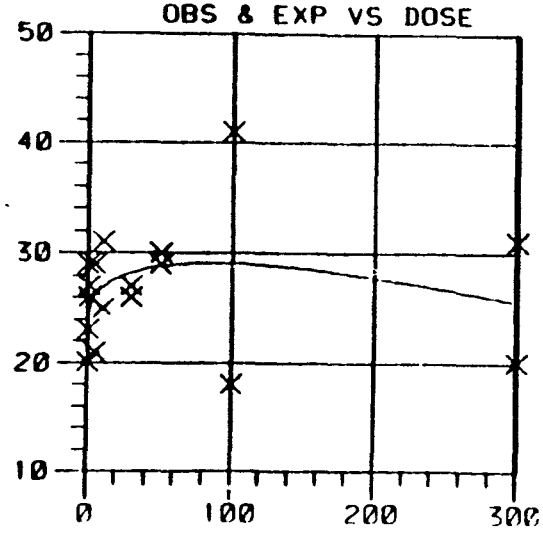
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	23	29	20	24.00	4.58
3.00*	UGS	250	270	255	258.33	10.41
1.00	UGS	26	27		26.50	.71
5.00	UGS	21	29		25.00	5.66
10.00	UGS	25	31		28.00	4.24
30.00	UGS	26	27		26.50	.71
50.00	UGS	30	29		29.50	.71
100.00	UGS	41	18		29.50	16.26
300.00	UGS	20	31		25.50	7.78

	B(0)	B(1)	B(2)	B(3)
ESTS	24.352	-.1211	.5262	.00166

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	15.07	9	.0891	-51.0685
ADEQUACY	.61	4	.9618	-51.3741
TOXICITY	.76	1	.3825	-51.7555
MUTAGENICITY	2.00	2	.3672	-52.3760

AVERAGE SLOPE (NONLIN. MODEL) = 139
95% CONF. LIMITS = (034, 574)

AVERAGE SLOPE (LINEAR REGR.) = 080
95% CONF. LIMITS = (-.018, .178)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT GREEN NO. 3 - C.I. SOLVENT YELLOW NO. 33 MIXTURE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0001 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	42	31	43	38.67	6.66
.50*	UGS	740	825	817	794.00	46.94
1.00	UGS	37	40		38.50	2.12
5.00	UGS	61	45		53.00	11.31
10.00	UGS	29	32		30.50	2.12
30.00	UGS	56	48		52.00	5.66
50.00	UGS	45	53		49.00	5.66
100.00	UGS	50	48		49.00	1.41
300.00	UGS	50	56		53.00	4.24

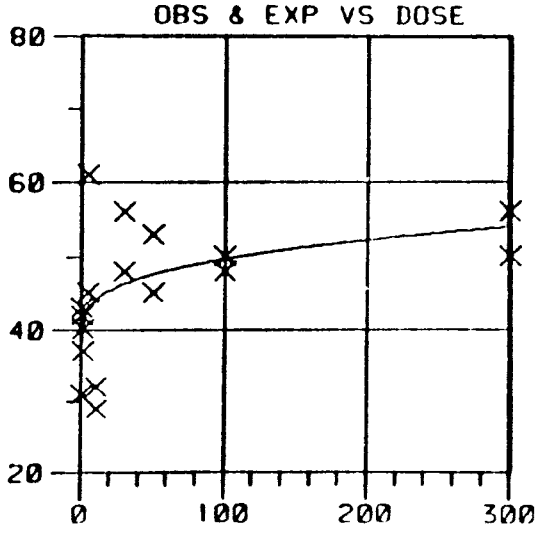
	B(0)	B(1)	B(2)
ESTS.	38.301	1.0715	.2941

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.62	9	.6765	-51.2029
ADEQUACY	15.58	5	.0081	-58.9945
MUTAGENICITY	8.58	2	.0137	-63.2862

AVERAGE SLOPE (NONLIN. MODEL) = .052
95% CONF. LIMITS = (.014, .194)

AVERAGE SLOPE (LINEAR REGR.) = .041
95% CONF. LIMITS = (-.003, .086)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: GBBA ON 03/30/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA100

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1179	1205	1180			1188.00	14.73
2-AA	RLA026	0.50	363	360	298			340.33	36.69
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	105	101	103			103.00	2.00
	-	100.000	103	134	97			111.33	19.86
BMGS-84-0001									
	RLA026	1.00	133	131				132.00	1.41
	RLA026	5.00	115	132				123.50	12.02
	RLA026	10.00	137	156				146.50	13.44
	RLA026	30.00	172	200				186.00	19.80
	RLA026	50.00	170	162				166.00	5.66
	RLA026	100.00	146	161				153.50	10.61
	RLA026	300.00	149	138				143.50	7.78
	RLA026	500.00	132	130				131.00	1.41
	RLA026	1000.00	125	135				130.00	7.07
	-	1.00	129	113				121.00	11.31
	-	5.00	141	141				141.00	0.00
	-	10.00	120	144				132.00	16.97
	-	30.00	154	166				160.00	8.49
	-	50.00	151	154				152.50	2.12
	-	100.00	142	141				141.50	0.71
	-	300.00	129	135				132.00	4.24
	-	500.00	139	108				123.50	21.92
	-	1000.00	93	114				102.50	13.44

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-GGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	105	101	103	103.00	2.00
.50*	UGS	363	360	298	340.33	36.69
1.00	UGS	133	131		132.00	1.41
5.00	UGS	115	132		123.50	12.02
10.00	UGS	137	156		146.50	13.44
30.00	UGS	172	200		186.00	19.80
50.00	UGS	170	162		166.00	5.66
100.00	UGS	146	161		153.50	10.61
300.00	UGS	149	138		143.50	7.78

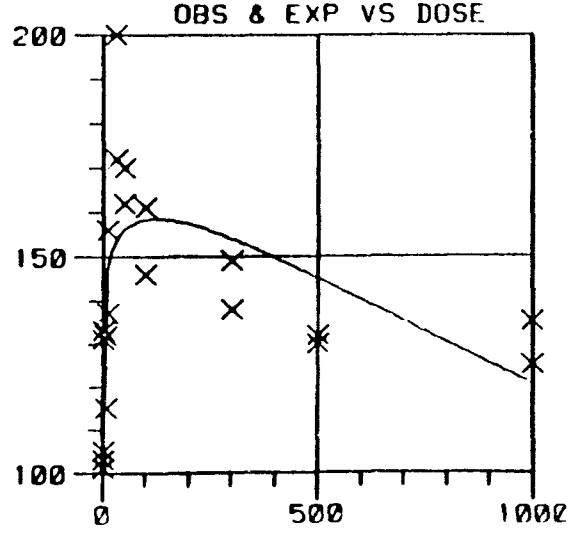
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	102.328	3.4880	.1439	.00046

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.35	11	.8490	-74.1956
ADEQUACY	25.88	6	.0002	-87.1344
TOXICITY	17.29	1	.0000	-95.7803
MUTAGENICITY	52.69	2	.0000	-113.4797

AVERAGE SLOPE (NONLIN. MODEL) = 1.779
95% CONF. LIMITS = (.516, 6.129)

AVERAGE SLOPE (LINEAR REGR.) = 2.471
95% CONF. LIMITS = (1.699, 3.242)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	103	134	97	111.33	19.86
3.00*	UGS	1179	1205	1180	1188.00	14.73
1.00	UGS	129	113		121.00	11.31
5.00	UGS	141	141		141.00	.00
10.00	UGS	120	144		132.00	16.97
30.00	UGS	154	166		160.00	8.49
50.00	UGS	151	154		152.50	2.12
100.00	UGS	142	141		141.50	.71
300.00	UGS	129	135		132.00	4.24

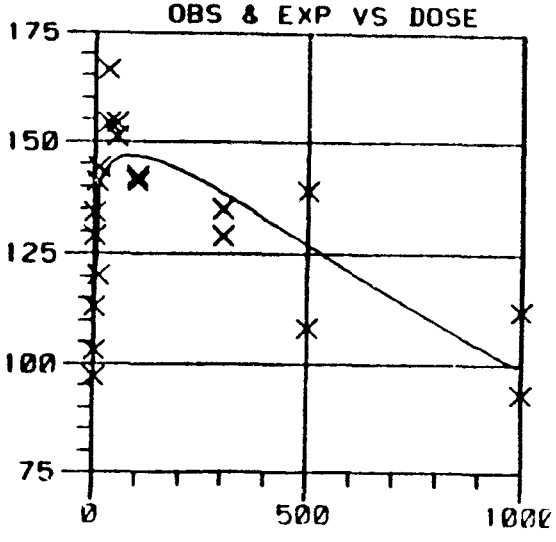
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	110.469	2.9785	.1807	.00059

TEST	CHI-SQUARE	DF	P	LOG L
POISSON	15.59	11	.1205	-78.5701
ADEQUACY	7.81	6	.2525	-82.4743
TOXICITY	29.90	1	.0000	-97.4251
MUTAGENICITY	25.98	2	.0000	-95.4635

AVERAGE SLOPE (NONLIN. MODEL) = 1.212
95% CONF. LIMITS = (.377, 1.674)

AVERAGE SLOPE (LINEAR REGR.) = 1.428
95% CONF. LIMITS = (.616, 2.241)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA100

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1253	1350	1320			1307.67	49.66
2-AA	RLA026	0.50	953	814	842			869.00	74.28
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	115	125	114			118.00	6.08
	-	100.000	109	102	110			107.00	4.36
BMGS-34-0032									
	RLA026	1.00	132	151				141.50	13.44
	RLA026	5.00	144	132				138.00	8.49
	RLA026	10.00	153	149				151.00	2.83
	RLA026	30.00	174	153				163.50	14.85
	RLA026	50.00	146	174				160.00	19.80
	RLA026	100.00	167	151				159.00	11.31
	RLA026	300.00	156	135				145.50	14.85
	-	1.00	130	130				130.00	0.00
	-	5.00	112	129				120.50	12.02
	-	10.00	128	112				120.00	11.31
	-	30.00	98	117				107.50	13.44
	-	50.00	126	132				129.00	4.24
	-	100.00	140	126				133.00	9.90
	-	300.00	111	118				114.50	4.95

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

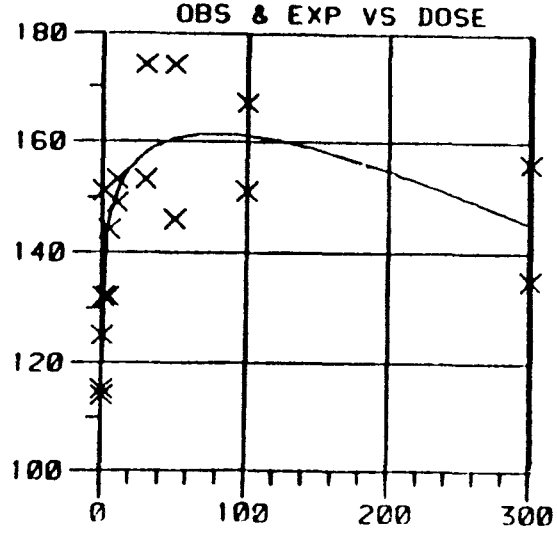
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	115	125	114	118.00	6.08
.50*	UGS	953	812	842	869.00	74.28
1.00	UGS	132	151		141.50	13.44
5.00	UGS	144	132		138.00	8.49
10.00	UGS	153	149		151.00	2.83
30.00	UGS	174	153		163.50	14.85
50.00	UGS	146	174		160.00	19.80
100.00	UGS	167	151		159.00	11.31
300.00	UGS	156	135		145.50	14.85

	B(0)	B(1)	B(2)	B(3)
ESTS.	118.182	2.9511	.2489	.00102

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.60	9	.4753	-62.1904
ADEQUACY	1.62	4	8061	-62.9979
TOXICITY	4.45	1	0350	-65.2215
MUTAGENICITY	26.13	2	0000	-76.0619

AVERAGE SLOPE (NONLIN. MODEL) = 1.486
95% CONF. LIMITS = (.760, 2.909)

AVERAGE SLOPE (LINEAR REGR.) = 1.245
95% CONF. LIMITS = (.553, 1.936)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

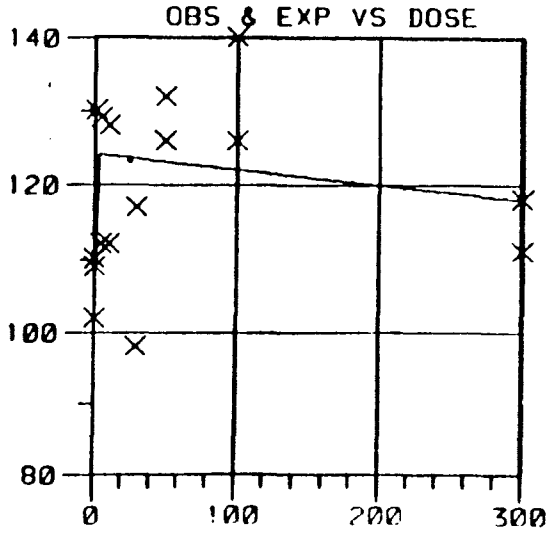
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	109	102	110	107.00	4.36
3.00*	UGS	1253	1350	1320	1307.67	49.66
1.00	UGS	130	130		130.00	.00
5.00	UGS	112	129		120.50	12.02
10.00	UGS	128	112		120.00	11.31
30.00	UGS	98	117		107.50	13.44
50.00	UGS	126	132		129.00	4.24
100.00	UGS	140	126		133.00	9.90
300.00	UGS	111	118		114.50	4.95

	B(0)	B(1)	B(2)	B(3)
ESTS.	106.793	2.8538	.0000	.00017

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	5.39	9	.7990	-58.9452
ADEQUACY	8.02	4	.0908	-62.9552
TOXICITY	.52	1	.4719	-63.2139
MUTAGENICITY	5.24	2	.0730	-65.5729

AVERAGE SLOPE (NONLIN MODEL) = .347
95% CONF. LIMITS = (.041, 2.972)

AVERAGE SLOPE (LINEAR REGR.) = .129
95% CONF. LIMITS = (- 239, .497)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: PLATE TEST - PREINCUBATION STRAIN: TA100

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	5.00	1227	1278	1267			1257.33	26.84
2-AA	RLAC26	0.50	354	307	337			332.67	23.80
NEG CONTROL									
DIMETHYLSULF	RLAC26	100.000	109	117	115			113.33	4.73
	-	100.000	144	132	127			134.33	8.74
SMGS-84-0002									
	RLAC26	1.00	129	133				131.00	2.83
	RLAC26	5.00	132					132.00	0.00
	RLAC26	10.00	151	140				145.50	7.78
	RLAC26	30.00	156	180				168.00	16.97
	RLAC26	50.00	194	203				198.50	6.36
	RLAC26	100.00	155	154				154.50	0.71
	RLAC26	300.00	172	137				154.50	24.75
	-	1.00	111	129				120.00	12.73
	-	5.00	111	137				124.00	18.39
	-	10.00	100	133				116.50	23.33
	-	30.00	139	101				120.00	26.87
	-	50.00	194	125				159.50	48.79
	-	100.00	103	110				106.50	4.95
	-	300.00	123	115				119.00	5.66

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

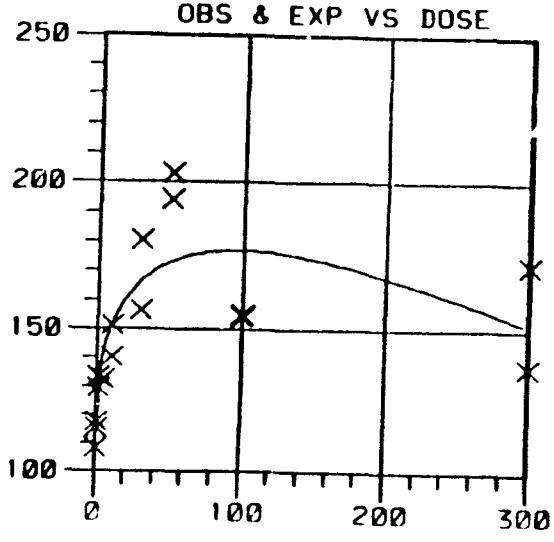
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	108	117	115	113.33	4.73
.50*	UGS	354	307	337	332.67	23.80
1.00	UGS	129	133		131.00	2.83
5.00	UGS	132			132.00	.00
10.00	UGS	151	140		145.50	7.78
30.00	UGS	156	180		168.00	16.97
50.00	UGS	194	203		198.50	6.36
100.00	UGS	155	154		154.50	.71
300.00	UGS	172	137		154.50	24.75

	B(0)	B(1)	B(2)	B(3)
ESTS.	112.794	2.8208	.3898	.00191

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.76	8	.5631	-57.9736
ADEQUACY	14.72	4	.0053	-65.3314
TOXICITY	12.75	1	.0004	-71.7061
MUTAGENICITY	55.73	2	.0000	-93.1984

AVERAGE SLOPE (NONLIN. MODEL) = 1.543
95% CONF. LIMITS = (1.022, 2.329)

AVERAGE SLOPE (LINEAR REGR.) = 1.548
95% CONF. LIMITS = (1.250, 1.846)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

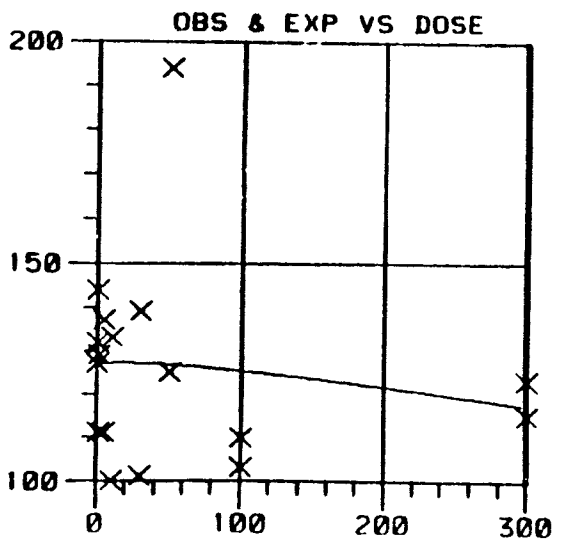
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	144	132	127	134.33	8.74
3.00*	UGS	1227	1278	1267	1257.33	26.84
1.00	UGS	111	129		120.00	12.73
5.00	UGS	111	137		124.00	18.38
10.00	UGS	100	133		116.50	23.33
30.00	UGS	139	101		120.00	26.87
50.00	UGS	194	125		159.50	48.79
100.00	UGS	103	110		106.50	4.95
300.00	UGS	123	115		119.00	5.66

	B(0)	B(1)	B(2)	B(3)
ESTS.	126.876	-1.4883	.7835	.00074

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	31.33	9	.0003	-72.3402
ADEQUACY	26.56	4	.0000	-85.6211
TOXICITY	1.30	1	.2534	-86.2732
MUTAGENICITY	.02	2	.9905	-85.6306

AVERAGE SLOPE (NONLIN. MODEL) = .066
95% CONF. LIMITS = (.000, ********)

AVERAGE SLOPE (LINEAR REGR.) = -.033
95% CONF. LIMITS = (-.151, .086)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLA027	30.00	1111	984	1009			1034.67	67.28
	-	0.50	349	335	382			355.33	24.13
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	108	109	100			105.67	4.93
	-	100.000	57	40	42			46.33	9.29
CMGS-84-0002									
	RLA027	10.00	100	100				100.00	0.00
	RLA027	30.00	144	162				153.00	12.73
	RLA027	50.00	186	187				186.50	0.71
	RLA027	100.00	213	190				201.50	16.26
	RLA027	300.00	188	206				197.00	12.73
	-	10.00	85	101				93.00	11.31
	-	30.00	113	91				102.00	15.56
	-	50.00	113	110				111.50	2.12
	-	100.00	74	100				87.00	16.38
	-	300.00	98	113				105.50	13.44

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1C2

+RLAC27

POSITIVE CONTROL USED WAS DANTHRON. DYES START TO PRECIPITATE OUT OF SOL UTION AT THE 3CCUG DOSE.

-

POSITIVE CONTROL USED WAS MITOMYCIN C.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/01/84 TECHNICIAN: MJK

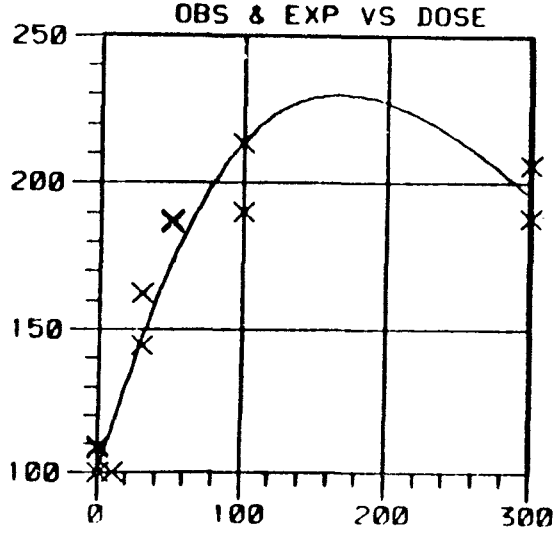
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	108	109	100	105.67	4.93
30.00*	UGS	1111	984	1009	1034.67	67.28
10.00	UGS	100	100		100.00	.00
30.00	UGS	144	162		153.00	12.73
50.00	UGS	186	187		186.50	.71
100.00	UGS	213	190		201.50	16.26
300.00	UGS	188	206		197.00	12.73

	B(0)	B(1)	B(2)	B(3)
ESTS.	101.044	5856	1.0826	.00532

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.66	7	.8183	-46.2231
ADEQUACY	9.62	2	.0081	-51.0330
TOXICITY	27.39	1	.0000	-64.7274
MUTAGENICITY	149.87	2	.0000	-125.9691

AVERAGE SLOPE (NONLIN. MODEL) = 2.628
95% CONF. LIMITS = (1.746, 3.955)

AVERAGE SLOPE (LINEAR REGR.) = 1.073
95% CONF. LIMITS = (.756, 1.390)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	57	40	42	46.33	9.29
.50*	UGS	349	335	382	355.33	24.13
10.00	UGS	85	101		93.00	11.31
30.00	UGS	113	91		102.00	15.56
50.00	UGS	113	110		111.50	2.12
100.00	UGS	74	100		87.00	18.38
300.00	UGS	96	115		105.50	13.44

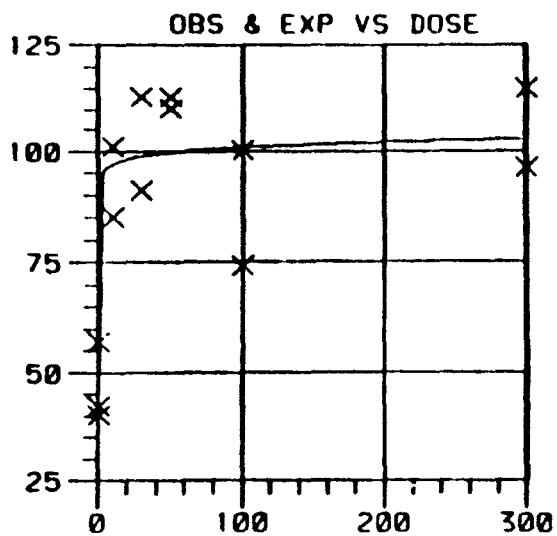
	B(0)	B(1)	B(2)
ESTS.	46.332	3.8521	.0316

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	13.11	7	.0694	-47.1769
ADEQUACY	7.38	3	.0607	-50.8666
MUTAGENICITY	87.17	2	.0000	-94.4495

AVERAGE SLOPE (NONLIN. MODEL) = .188
95% CONF. LIMITS = (.151, .234)

AVERAGE SLOPE (LINEAR REGR.) = .106
95% CONF. LIMITS = (-.032, .244)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLAC27	30.00		(4)1176	1208			1192.00	22.63
	-	0.50	1182	1164	1170			1173.33	7.57
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	257	234	239			243.33	12.10
	-	100.000	154	(4)	145			149.50	6.36
BMGS-84-0002									
	RLAC27	10.00	403	441				422.00	26.87
	RLAC27	30.00	538	464				501.00	52.33
	RLAC27	50.00	603	608				605.50	3.54
	RLAC27	100.00	640	665				652.50	17.68
	RLAC27	300.00	678	662				670.00	11.31
	-	10.00	341	350				345.50	6.36
	-	30.00	474	440				457.00	24.04
	-	50.00	506	424				465.00	57.98
	-	100.00	441	463				452.00	15.56
	-	300.00	485	517				501.00	22.63

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS Y-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/06/84

08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION

STRAIN: TA102

BACKGROUNDS:

(4)CONTAMINATED

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: 688A ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1G2

•RLAG27

DANTHRON WAS USED AS THE POSITIVE CONTROL.

-

MITOMYCIN C WAS USED AS THE POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/08/84 TECHNICIAN: MJK

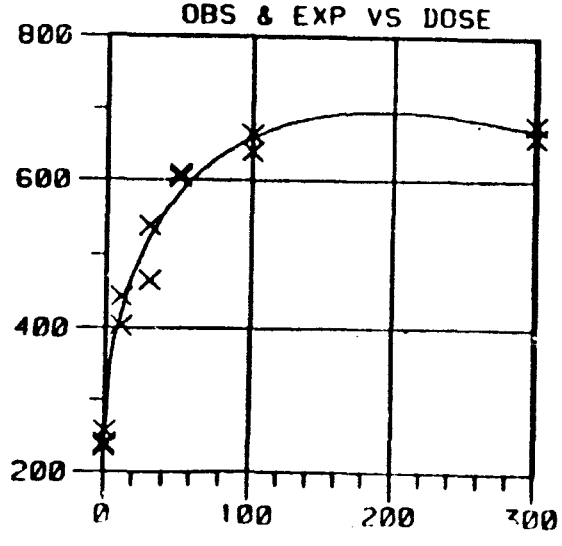
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UCS	257	234	239	243.33	12.10
30.00*	UCS		1176	1208	1192.00	22.63
10.00	UCS	403	441		422.00	26.87
30.00	UCS	538	464		501.00	52.33
50.00	UCS	603	608		605.50	3.54
100.00	UCS	640	665		652.50	17.68
300.00	UCS	678	662		670.00	11.31

	B(0)	B(1)	B(2)	B(3)
ESTS.	243.447	4.0857	.4853	.00192

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	9.07	7	.2477	-56.3735
ADEQUACY	3.93	2	.1404	-58.3371
TOXICITY	24.06	1	.0000	-70.3655
MUTAGENICITY	768.54	2	.0000	-442.0078

AVERAGE SLOPE (NONLIN. MODEL) = 5.559
95% CONF. LIMITS = (4.325, 7.145)

AVERAGE SLOPE (LINEAR REGR.) = 3.867
95% CONF. LIMITS = (2.480, 5.254)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/08/84 TECHNICIAN: MJK

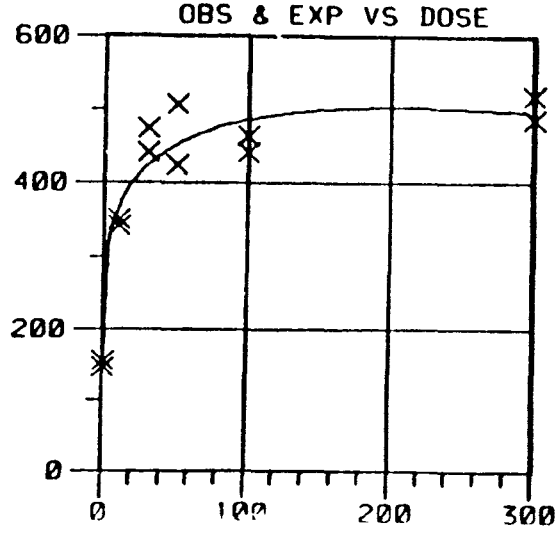
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	154		145	149.50	6.36
.50*	UGS	1182	1168	1170	1173.33	7.57
10.00	UGS	341	350		345.50	6.36
30.00	UGS	474	440		457.00	24.04
50.00	UGS	506	424		465.00	57.98
100.00	UGS	441	463		452.00	15.56
300.00	UGS	485	517		501.00	22.63

	B(0)	B(1)	B(2)	B(3)
ESTS.	149.142	4.8437	.2383	.00067

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	10.44	6	.1073	-51.6990
ADEQUACY	13.07	2	.0015	-58.2338
TOXICITY	5.90	1	.0152	-61.1818
MUTAGENICITY	534.73	2	.0000	-325.5995

AVERAGE SLOPE (NONLIN. MODEL) = 1.647
95% CONF. LIMITS = (1.329, 2.042)

AVERAGE SLOPE (LINEAR REGR.) = .667
95% CONF. LIMITS = (.052, 1.283)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/15/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	uGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLA027	0.50	1252	1377	1392	1370		1347.75	64.49
	-	30.00	1380	1320	1363			1361.00	35.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	264	264	266			264.67	1.15
	-	100.000	202	186	213			201.00	12.53
BMGS-34-0002									
	RLA027	1.00	283	292				287.50	6.36
	RLA027	5.00	317	377				347.00	42.43
	RLA027	10.00	403	341				372.00	43.84
	RLA027	30.00	549	522				535.50	19.09
	RLA027	50.00	698	649				673.50	34.65
	RLA027	100.00	711	707				709.00	2.83
	RLA027	300.00	777	701				739.00	53.74
	-	1.00	217	191				204.00	18.38
	-	5.00	300	280				290.00	14.14
	-	10.00	383	359				371.00	16.97
	-	30.00	417	413				415.00	2.83
	-	50.00	446	445				445.50	3.54
	-	100.00	463	461				462.00	1.41
	-	300.00	435	500				467.50	45.96

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

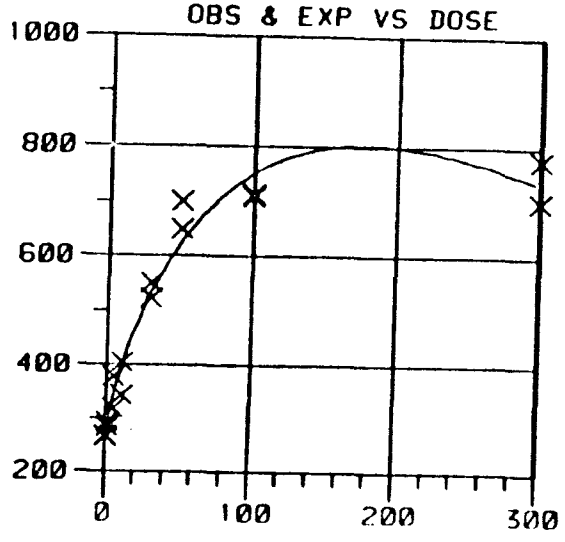
DOSE	UNITS	PLATE COUNTS				MEAN	S.D.
.00	UGS	264	264	266		264.67	1.16
.50*	UGS	1252	1377	1392	1370	1347.75	64.49
1.00	UGS	283	292			287.50	6.36
5.00	UGS	317	377			347.00	42.43
10.00	UGS	403	341			372.00	43.84
30.00	UGS	549	522			535.50	19.09
50.00	UGS	698	649			673.50	34.65
100.00	UGS	711	707			709.00	2.83
300.00	UGS	777	701			739.00	53.74

	B(0)	B(1)	B(2)	B(3)
ESTS.	261.427	3.3232	.7267	.00337

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	16.89	9	.0505	-75.8276
ADEQUACY	15.48	4	.0038	-83.5691
TOXICITY	100.28	1	.0000	-133.7075
MUTAGENICITY	1277.73	2	.0000	-722.4327

AVERAGE SLOPE (NONLIN. MODEL) = 5.838
95% CONF. LIMITS = (4.328, 7.873)

AVERAGE SLOPE (LINEAR REGR.) = 1.490
95% CONF. LIMITS = (.797, 2.184)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

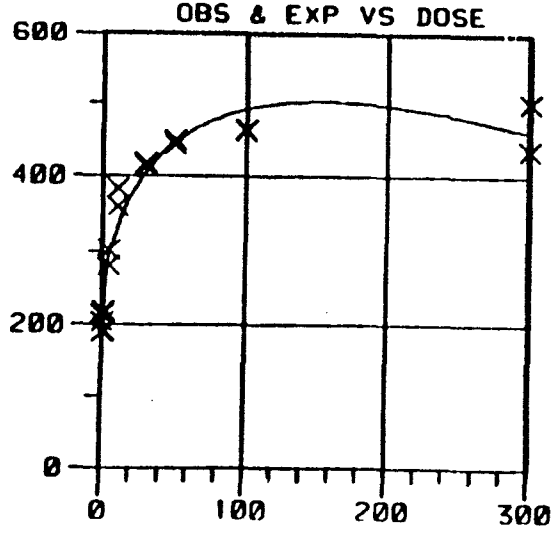
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	202	188	213	201.00	12.53
30.00*	UGS	1380	1320	1383	1361.00	35.54
1.00	UGS	217	191		204.00	18.38
5.00	UGS	300	280		290.00	14.14
10.00	UGS	383	359		371.00	16.97
30.00	UGS	417	413		415.00	2.83
50.00	UGS	448	443		445.50	3.54
100.00	UGS	463	461		462.00	1.41
300.00	UGS	435	500		467.50	45.96

	B(0)	B(1)	B(2)	B(3)
ESTS.	192.567	3.9137	.4608	.00219

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	9.26	9	.4140	-69.5208
ADEQUACY	27.87	4	.0000	-83.4568
TOXICITY	50.29	1	.0000	-108.6019
MUTAGENICITY	590.24	2	.0000	-378.5747

AVERAGE SLOPE (NONLIN. MODEL) = 2.312
95% CONF. LIMITS = (1.888, 2.830)

AVERAGE SLOPE (LINEAR REGR.) = .724
95% CONF. LIMITS = (.258, 1.191)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1C4

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-AA	RLAC27	3.00	2393	2440	2467			2433.33	37.45
OTHER POS	-	17.00	765	851	777			797.67	46.58
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	312	311	340			321.00	16.46
	-	100.000	262	323	271			285.33	32.93
BMGS-84-0002									
	RLAC27	10.00	485	467				476.00	12.73
	RLAC27	30.00	466	491				478.50	17.68
	RLAC27	50.00	483	465				474.00	12.73
	RLAC27	100.00	474	492				483.00	12.73
	RLAC27	300.00	341	346				343.50	3.54
	-	10.00	343	303				323.00	28.28
	-	30.00	290	295				292.50	3.54
	-	50.00	305	325				315.00	14.14
	-	100.00	323	319				321.00	2.83
	-	300.00	293	319				306.00	18.38

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50CUGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

POSITIVE CONTROL USED WAS METHYL GLYOXAL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	312	311	340	321.00	16.46
3.00*	UGS	2393	2440	2467	2433.33	37.45
10.00	UGS	485	467		476.00	12.73
30.00	UGS	466	491		478.50	17.68
50.00	UGS	483	465		474.00	12.73
100.00	UGS	474	492		483.00	12.73
300.00	UGS	341	346		343.50	3.54

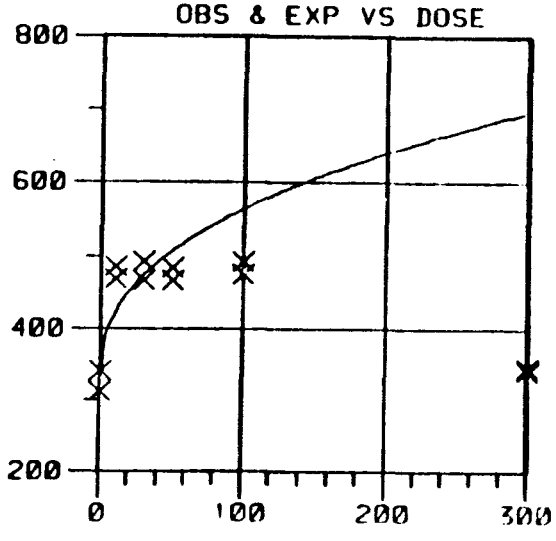
	B(0)	B(1)	B(2)
ESTS.	329.883	3.5885	.4055

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.40	7	.8462	-52.8098
ADEQUACY	483.88	3	.0000	-294.7511
MUTAGENICITY	.01	2	.9950	-136.4160

AVERAGE SLOPE (NONLIN. MODEL) = 3.535

AVERAGE SLOPE (LINEAR REGR.) = 2.715
95% CONF. LIMITS = (.694, 4.735)
WARNING. 4 PARAMETER MODEL DID NOT CONVERGE
WARNING. 3 PARAMETER MODEL DID NOT CONVERGE



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	262	323	271	285.33	32.93
17.00*	UGS	765	851	777	797.67	46.58
10.00	UGS	343	303		323.00	28.28
30.00	UGS	290	295		292.50	3.54
50.00	UGS	305	325		315.00	14.14
100.00	UGS	323	319		321.00	2.83
300.00	UGS	293	319		306.00	18.38

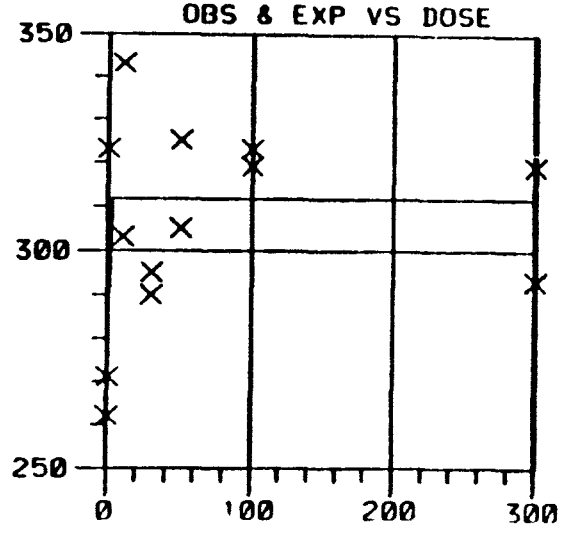
	B(0)	B(1)	B(2)
ESTS.	285.333	3.2723	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	11.88	7	.1044	-54.9980
ADEQUACY	4.05	3	.2559	-57.0243
MUTAGENICITY	5.25	2	.0723	-59.6507

AVERAGE SLOPE (NONLIN. MODEL) = .527
95% CONF. LIMITS = (.267, 1.042)

AVERAGE SLOPE (LINEAR REGR.) = .344
95% CONF. LIMITS = (-.574, 1.262)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-AA	RLA027	3.00	2053	2002	2086			2047.00	42.32
OTHER POS	-	50.00	1810	1718	1760			1769.33	46.92
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	363	402	375			380.00	19.97
	-	100.000	277	266	279			274.00	7.00
BMGS-84-0002									
	RLA027	1.00	329	301				315.00	19.80
	RLA027	5.00	361	358				359.50	2.12
	RLA027	10.00	442	483				462.50	28.99
	RLA027	30.00	533	502				517.50	21.92
	RLA027	50.00	500	536				518.00	25.46
	RLA027	100.00	476	500				488.00	16.97
	RLA027	300.00	452	451				451.50	0.71
	-	1.00	258	281				269.50	16.26
	-	5.00	317	271				294.00	32.53
	-	10.00	324	314				319.00	7.07
	-	30.00	324	317				320.50	4.95
	-	50.00	347	299				323.00	33.94
	-	100.00	343	326				334.50	12.02
	-	300.00	336	340				338.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

METHYL GLYOXAL WAS USED AS A POSITIVE CONTROL.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	363	402	375	380.00	19.97
3.00*	UGS	2053	2002	2086	2047.00	42.32
1.00	UGS	329	301		315.00	19.80
5.00	UGS	361	358		359.50	2.12
10.00	UGS	442	483		462.50	28.99
30.00	UGS	533	502		517.50	21.92
50.00	UGS	500	536		518.00	25.46
100.00	UGS	476	500		488.00	16.97
300.00	UGS	452	451		451.50	.71

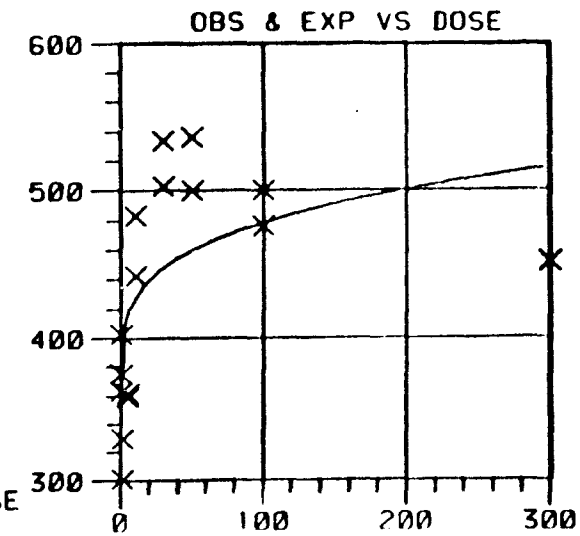
	B(0)	B(1)	B(2)
ESTS.	360.889	3.6116	.2501

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	7.94	9	.5397	-71.0742
ADEQUACY	114.24	5	.0000	-128.1922
MUTAGENICITY	84.98	2	.0000	-170.6841

AVERAGE SLOPE (NONLIN. MODEL) = 2.890
95% CONF. LIMITS = (2.367, 3.527)

AVERAGE SLOPE (LINEAR REGR.) = 5.704
95% CONF. LIMITS = (3.429, 7.978)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/08/84 TECHNICIAN: MJK

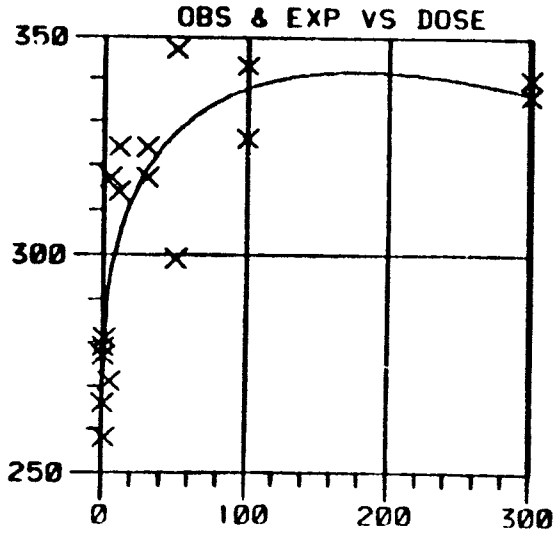
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	277	266	279	274.00	7.00
50.00*	UGS	1810	1718	1780	1769.33	46.92
1.00	UGS	258	281		269.50	16.26
5.00	UGS	317	271		294.00	32.53
10.00	UGS	324	314		319.00	7.07
30.00	UGS	324	317		320.50	4.95
50.00	UGS	347	299		323.00	33.94
100.00	UGS	343	326		334.50	12.02
300.00	UGS	336	340		338.00	2.83

	B(0)	B(1)	B(2)	B(3)
ESTS.	270.935	2.5639	.4248	.00072

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	9.19	9	.4196	-68.8684
ADEQUACY	3.37	4	.4978	-70.5536
TOXICITY	2.19	1	.1385	-71.6508
MUTAGENICITY	33.03	2	.0000	-87.0686

AVERAGE SLOPE (NONLIN. MODEL) = 3.453
95% CONF. LIMITS = (.908, 13.129)

AVERAGE SLOPE (LINEAR REGR.) = 4.781
95% CONF. LIMITS = (2.229, 7.333)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF [illegible] DYE YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1535

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1021	1031	959			1003.67	39.00
2-AA	RLA027	3.00	153	164	132			149.67	16.26
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	26	24	29			26.33	2.52
	-	100.000	37	44	33			38.00	5.57
EMGS-84-0002									
	RLA027	10.00	27	28				27.50	0.71
	RLA027	30.00	18	25				21.50	4.95
	RLA027	50.00	17	21				19.00	2.83
	RLA027	100.00	19	15				17.00	2.83
	RLA027	300.00	25	18				21.50	4.95
	-	10.00	48	36				42.00	8.49
	-	30.00	31	28				29.50	2.12
	-	50.00	24	32				28.00	5.66
	-	100.00	36	36				36.00	0.00
	-	300.00	55	37				46.00	12.73

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS :-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/01/84 TECHNICIAN: MJK

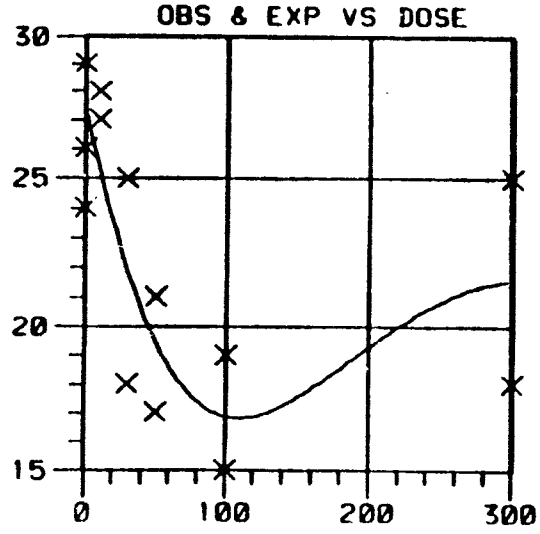
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	26	24	29	26.33	2.52
3.00*	UGS	153	164	132	149.67	16.26
10.00	UGS	27	28		27.50	.71
30.00	UGS	18	25		21.50	4.95
50.00	UGS	17	21		19.00	2.83
100.00	UGS	19	15		17.00	2.83
300.00	UGS	25	18		21.50	4.95

	B(0)	B(1)	B(2)	B(3)
ESTS.	27.364	-10.4407	2.7614	.00789

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.67	7	.8169	-33.9265
ADEQUACY	.52	2	.7705	-34.1873
TOXICITY	7.68	1	.0056	-38.0263
MUTAGENICITY	6.33	2	.0422	-37.3520

AVERAGE SLOPE (NONLIN. MODEL) = .674
95% CONF. LIMITS = (.001, 823.050)

AVERAGE SLOPE (LINEAR REGR.) = -.015
95% CONF. LIMITS = (-.040, .010)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	37	44	33	38.00	5.57
3.00*	UGS	1021	1031	959	1003.67	39.00
10.00	UGS	48	36		42.00	8.49
30.00	UGS	31	28		29.50	2.12
50.00	UGS	24	32		28.00	5.66
100.00	UGS	36	36		36.00	.00
300.00	UGS	55	37		46.00	12.73

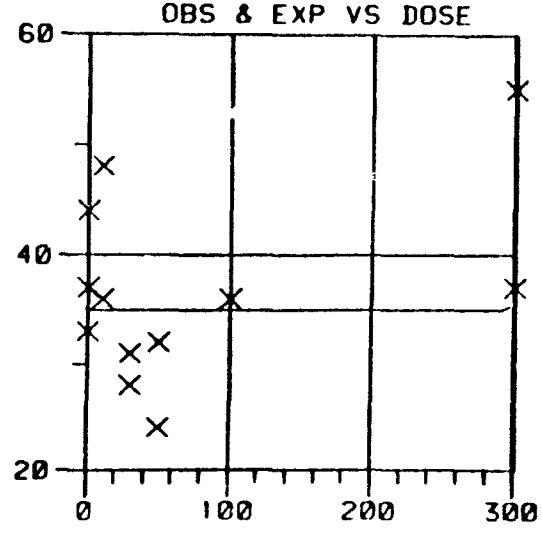
	B(0)	B(1)	B(2)
ESTS.	35.013	**********	322.3196

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.16	7	.3184	-39.3381
ADEQUACY	8.27	3	.0407	-43.4740
MUTAGENICITY	5.24	2	.0728	-46.0935

AVERAGE SLOPE (NONLIN. MODEL) = .037
95% CONF. LIMITS = (.013, .099)

AVERAGE SLOPE (LINEAR REGR.) = .032
95% CONF. LIMITS = (-.011, .075)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1535

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1003	979	1014			998.67	17.93
2-AA	RLA027	3.00	89	103	109			100.33	10.26
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	25	24	18			22.33	3.79
	-	100.000	43	26	28			32.33	9.29
BMGS-84-0002									
	RLA027	10.00	20	20				20.00	0.00
	RLA027	30.00	9	18				13.50	6.36
	RLA027	50.00	15	14				14.50	0.71
	RLA027	100.00	20	17				18.50	2.12
	RLA027	300.00	10	16				13.00	4.24
	-	10.00	25	31				28.00	4.24
	-	30.00	32	36				34.00	2.83
	-	50.00	36	33				34.50	2.12
	-	100.00	41	50				45.50	6.36
	-	300.00	29	34				31.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/08/84 TECHNICIAN: MJK

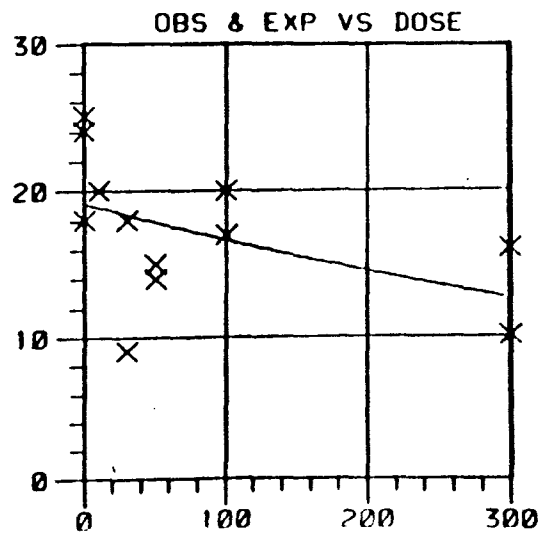
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	25	24	18	22.33	3.79
3.00*	UGS	89	103	109	100.33	10.26
10.00	UGS	20	20		20.00	.00
30.00	UGS	9	18		13.50	6.36
50.00	UGS	15	14		14.50	.71
100.00	UGS	20	17		18.50	2.12
300.00	UGS	10	16		13.00	4.24

	B(0)	B(1)	B(2)	B(3)
ESTS.	19.124*********		.0000	.00139

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	5.95	7	.5461	-33.3539
ADEQUACY	6.25	2	.0440	-36.4774
TOXICITY	3.84	1	.0500	-38.3974
MUTAGENICITY	.00	21	.0000	-36.4774

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = -.040
95% CONF. LIMITS = (-.116, .037)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/08/84 TECHNICIAN: MJK

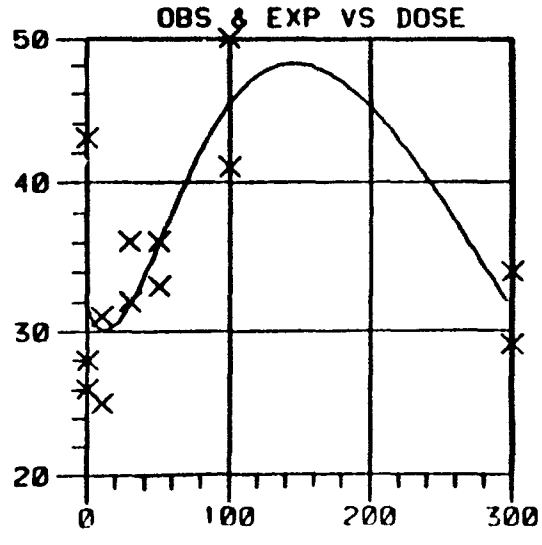
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	43	26	28	32.33	9.29
3.00*	UGS	1003	979	1014	998.67	17.90
10.00	UGS	25	31		28.00	4.24
30.00	UGS	32	36		34.00	2.83
50.00	UGS	36	33		34.50	2.12
100.00	UGS	41	50		45.50	6.36
300.00	UGS	29	34		31.50	3.54

	B(0)	B(1)	B(2)	B(3)
ESTS.	31.624	-3.4714	1.7390	.01016

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	7.64	7	.3658	-38.5172
ADEQUACY	.75	2	.6858	-38.8944
TOXICITY	8.15	1	.0043	-42.9689
MUTAGENICITY	9.32	2	.0095	-43.5539

AVERAGE SLOPE (NONLIN. MODEL) = .934
95% CONF. LIMITS = (.187, 4.659)

AVERAGE SLOPE (LINEAR REGR.) = .143
95% CONF. LIMITS = (.045, .241)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: GBdA GN 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1537

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
9-AA	-	100.00	1129	1111	880			1040.00	138.86
2-AA	RLA027	3.00	304	380	343			342.33	38.00
NEG CONTROL									
DIMETHYLSULF	RLA027	100.0000	17	15	27			19.67	6.43
	-	100.0000	14	18	8			13.33	5.03
BMGS-84-0002									
	RLA027	10.00	19	29				23.50	7.78
	RLA027	30.00	25	25				25.00	0.00
	RLA027	50.00	28	33				30.50	3.54
	RLA027	100.00	32	25				28.50	4.95
	-	10.00	15	12				13.50	2.12
	-	30.00	13	13				13.00	0.00
	-	50.00	15	19				17.00	1.41
	-	100.00	25	17				21.00	5.66
	-	300.00	25	23				24.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	17	15	27	19.67	6.43
3.00*	UGS	304	380	343	342.33	38.00
10.00	UGS	18	29		23.50	7.78
30.00	UGS	25	25		25.00	.00
50.00	UGS	28	33		30.50	3.54
100.00	UGS	32	25		28.50	4.95

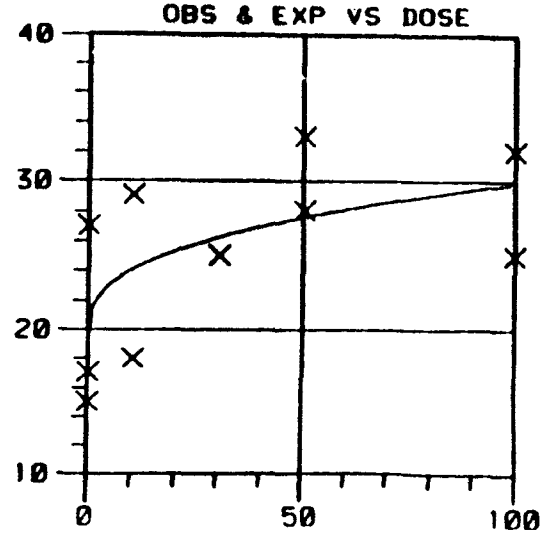
	B(0)	B(1)	B(2)
ESTS.	19.603	.6530	.3628

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.05	6	.2347	-31.6221
ADEQUACY	.87	2	.6484	-32.0554
MUTAGENICITY	6.20	2	.0451	-35.1536

AVERAGE SLOPE (NONLIN. MODEL) = .159
95% CONF. LIMITS = (.064, .397)

AVERAGE SLOPE (LINEAR REGR.) = .200
95% CONF. LIMITS = (.035, .365)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: MJK

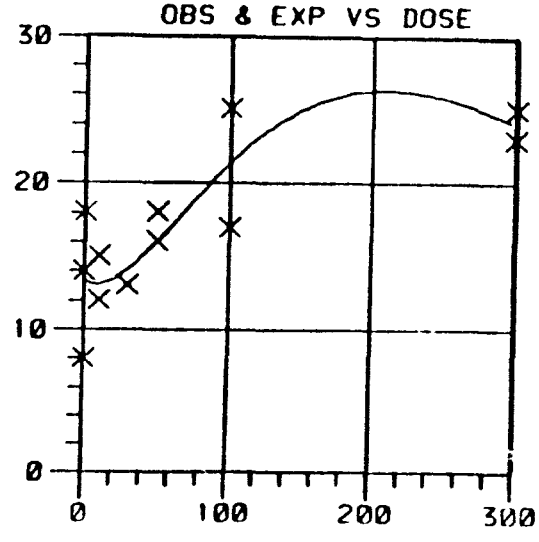
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	14	18	8	13.33	5.03
100.00*	UGS	1129	1111	880	1040.00	138.86
10.00	UGS	15	12		13.50	2.12
30.00	UGS	13	13		13.00	.00
50.00	UGS	18	16		17.00	1.41
100.00	UGS	25	17		21.00	5.66
300.00	UGS	25	23		24.00	1.41

	B(0)	B(1)	B(2)	B(3)
ESTS.	13.378	-4.2615	1.6609	.00702

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	5.86	7	.5564	-33.0506
ADEQUACY	.33	2	.8486	-33.2147
TOXICITY	1.26	1	.2615	-33.8451
MUTAGENICITY	13.06	2	.0015	-39.7471

AVERAGE SLOPE (NONLIN. MODEL) = .296
95% CONF. LIMITS = (.009, 9.754)

AVERAGE SLOPE (LINEAR REGR.) = .080
95% CONF. LIMITS = (.024, .137)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1537

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
9-AA	-	100.00	512	605	653			523.33	76.63
2-AA	RLA027	3.00	314	316	311			313.67	2.52
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	20	18	16			18.00	2.00
	-	100.000	12	7	17			12.00	5.00
BMGS-34-0002									
	RLA027	10.00	26	17				21.50	6.36
	RLA027	30.00	27	33				30.00	4.24
	RLA027	50.00	36	40				38.00	2.83
	RLA027	100.00	24	31				27.50	4.95
	RLA027	300.00	31	26				28.50	3.54
	-	10.00	12	12				12.00	0.00
	-	30.00	29	19				24.00	7.07
	-	50.00	28	26				27.00	1.41
	-	100.00	18	19				18.50	0.71
	-	300.00	20	15				17.50	3.54

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50.0UGS

T=-TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: MJK

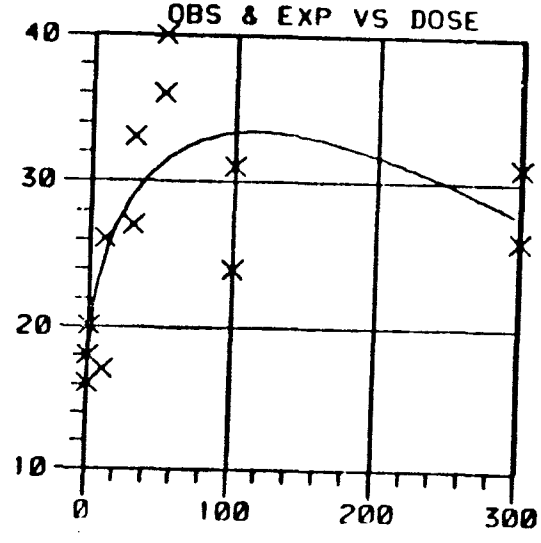
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	20	18	16	18.00	2.00
3.00*	UGS	314	316	311	313.67	2.52
10.00	UGS	26	17		21.50	6.36
30.00	UGS	27	33		30.00	4.24
50.00	UGS	36	40		38.00	2.83
100.00	UGS	24	31		27.50	4.95
300.00	UGS	31	26		28.50	3.54

	B(0)	B(1)	B(2)	B(3)
ESTS.	17.685	.7996	.5412	.00292

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.47	7	.7245	-35.2934
ADEQUACY	5.86	2	.0535	-38.2214
TOXICITY	2.93	1	.0869	-39.6867
MUTAGENICITY	15.94	2	.0003	-46.1924

AVERAGE SLOPE (NONLIN. MODEL) = .370
95% CONF. LIMITS = (.164, .833)

AVERAGE SLOPE (LINEAR REGR.) = .403
95% CONF. LIMITS = (.291, .515)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/08/84 TECHNICIAN: MJK

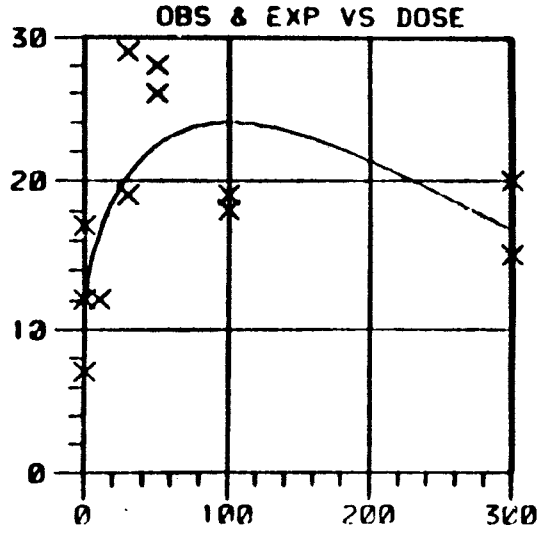
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	12	7	17	12.00	5.00
100.00*	UGS	512	605	453	523.33	76.63
10.00	UGS	12	12		12.00	.00
30.00	UGS	29	19		24.00	7.07
50.00	UGS	28	26		27.00	1.41
100.00	UGS	18	19		18.50	.71
300.00	UGS	20	15		17.50	3.54

	B(0)	B(1)	B(2)	B(3)
ESTS.	11.475	.2026	.6690	.00464

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	7.07	7	.4221	-33.9597
ADEQUACY	8.50	2	.0143	-38.2095
TOXICITY	5.42	1	.0199	-40.9205
MUTAGENICITY	15.40	2	.0005	-45.9120

AVERAGE SLOPE (NONLIN. MODEL) = .335
95% CONF. LIMITS = (.176, .640)

AVERAGE SLOPE (LINEAR REGR.) = .335
95% CONF. LIMITS = (.185, .485)



A-101

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	5.00	476	432	512			473.33	40.07
2-AA	RLAC27	0.50	704	729	692			708.33	18.88
NEG CONTROL									
DIMETHYLSULF	RLAC27	100.000	26	25	24			25.00	1.00
	-	100.000	17	13	18			16.00	2.65
BMGS-84-0002									
	RLAC27	10.00	44	36				40.00	5.66
	RLAC27	30.00	28	29				28.50	0.71
	RLAC27	50.00	31	27				29.00	2.83
	RLAC27	100.00	36	29				32.50	4.95
	RLAC27	300.00	28	38				33.00	7.07
	-	10.00	28	21				24.50	4.95
	-	30.00	13	8				10.50	3.54
	-	50.00	15	21				18.00	4.24
	-	100.00	18	17				17.50	0.71
	-	300.00	19	29				24.00	7.07

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 5000GS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-GGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	26	25	24	25.00	1.00
.50*	UGS	704	729	692	708.33	18.88
10.00	UGS	44	36		40.00	5.66
30.00	UGS	28	29		28.50	.71
50.00	UGS	31	27		29.00	2.83
100.00	UGS	36	29		32.50	4.95
300.00	UGS	28	38		33.00	7.07

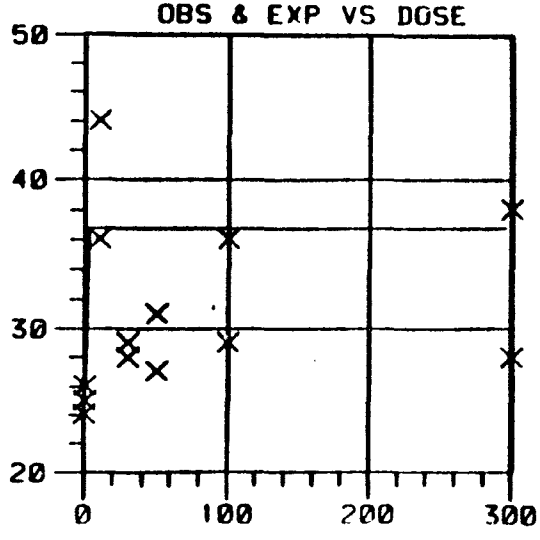
	B(0)	B(1)	B(2)
ESTS.	25.000	2.4592	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.44	7	.8413	-35.8944
ADEQUACY	9.79	3	.0204	-40.7917
MUTAGENICITY	.01	2	.9950	-40.6844

AVERAGE SLOPE (NONLIN. MODEL) = .117

AVERAGE SLOPE (LINEAR REGR.) = .024
95% CONF. LIMITS = (-.082, .130)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	17	13	18	16.00	2.65
3.00*	UGS	476	432	512	473.33	40.07
10.00	UGS	28	21		24.50	4.95
30.00	UGS	13	8		10.50	3.54
50.00	UGS	15	21		18.00	4.24
100.00	UGS	18	17		17.50	.71
300.00	UGS	19	29		24.00	7.07

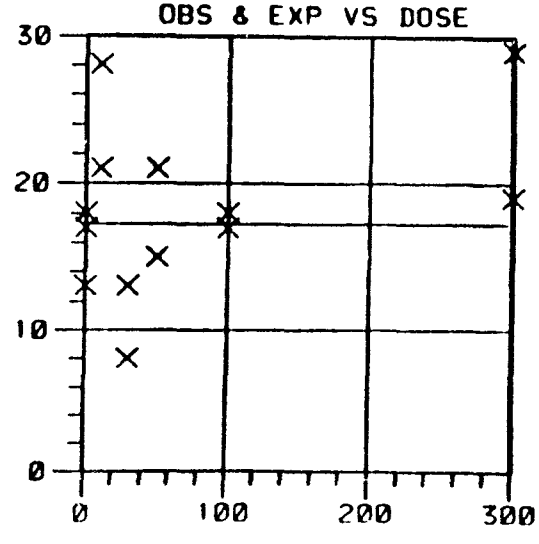
	B(0)	B(1)	B(2)
ESTS.	17.187******		

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.18	7	.5192	-33.6811
ADEQUACY	11.88	3	.0078	-39.6216
MUTAGENICITY	3.99	2	.1357	-41.6189

AVERAGE SLOPE (NONLIN. MODEL) = .023

AVERAGE SLOPE (LINEAR REGR.) = .022
95% CONF. LIMITS = (-.008, .052)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA153o

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	5.00	502	604	528			544.67	53.00
2-AA	RLA027	0.50	816	807	828			817.00	10.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	60	62	73			71.67	9.07
	-	100.000	21	18	19			19.33	1.53
BMGS-84-0002									
	RLA027	10.00	31	31				31.00	0.00
	RLA027	30.00	37	27				32.00	7.07
	RLA027	50.00	38	39				38.50	0.71
	RLA027	100.00	37	28				32.50	6.36
	RLA027	300.00	28	28				28.00	0.00
	-	10.00	8	13				10.50	3.54
	-	30.00	9	14				11.50	3.54
	-	50.00	14	29				21.50	10.61
	-	100.00	17	19				18.00	1.41
	-	300.00	12	18				15.00	4.24

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY CHK : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: GBBA ON 06/08/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

*RLAC27

SPONTANEOUS COUNT FOR 1538 IS HIGH. SMALL COLONIES ON THE PLATE ACCOUNTED FOR THE HIGH COUNT. SMALL COLONIES WERE SALMONELLA.

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/08/84 TECHNICIAN: MJK

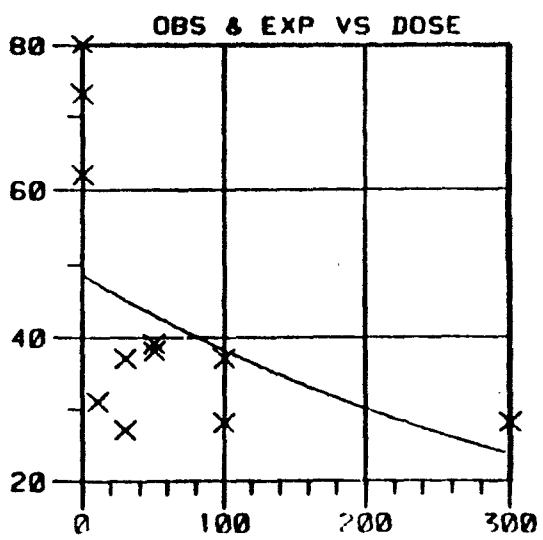
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	80	62	73	71.67	9.07
.50*	UGS	816	807	828	817.00	10.54
10.00	UGS	31	31		31.00	.00
30.00	UGS	37	27		32.00	7.07
50.00	UGS	38	39		38.50	.71
100.00	UGS	37	28		32.50	6.36
300.00	UGS	28	28		28.00	.00

	B(0)	B(1)	B(2)	B(3)
ESTS.	48.430	-82.0591	.0000	.00240

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	5.12	7	.6454	-38.2959
ADEQUACY	54.57	2	.0000	-65.5830
TOXICITY	167.22	1	.0000	-149.1949
MUTAGENICITY	.01	2	.9950	-65.5829

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = -.579
95% CONF. LIMITS = (-1.160, .001)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/08/84 TECHNICIAN: MJK

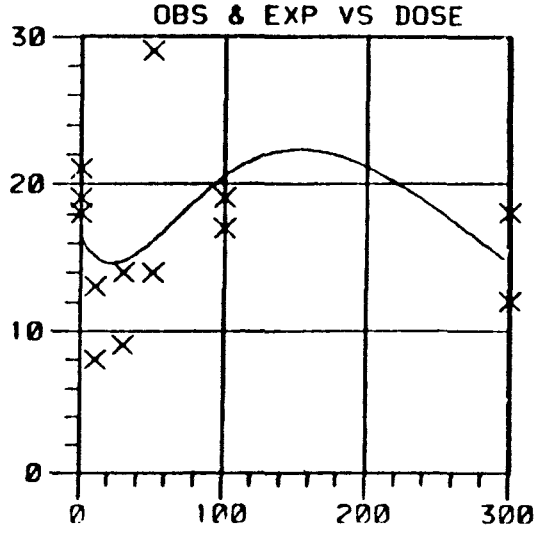
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	21	18	19	19.33	1.53
3.00*	UGS	502	604	528	544.67	53.00
10.00	UGS	8	13		10.50	3.54
30.00	UGS	9	14		11.50	3.54
50.00	UGS	14	29		21.50	10.61
100.00	UGS	17	19		18.00	1.41
300.00	UGS	12	18		15.00	4.24

	B(0)	B(1)	B(2)	B(3)
ESTS.	16.328	-5.2186	1.9665	.01119

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	9.06	7	.2482	-34.3809
ADEQUACY	10.12	2	.0063	-39.4434
TOXICITY	2.92	1	.0875	-40.9034
MUTAGENICITY	2.89	2	.2358	-40.8881

AVERAGE SLOPE (NONLIN. MODEL) = .237
95% CONF. LIMITS = (.067, .837)

AVERAGE SLOPE (LINEAR REGR.) = .044
95% CONF. LIMITS = (-.191, .280)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 06/20/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	492	504	551			515.67	31.18
2-AA	RLA027	0.50	668	714	737			706.33	35.13
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	45	39	36			40.00	4.58
	-	100.000	8	12	16			12.00	4.00
BMGS-84-0002									
	RLA027	10.00	31	28				29.50	2.12
	RLA027	30.00	31	28				29.50	2.12
	RLA027	50.00	39	43				41.00	2.83
	RLA027	100.00	36	32				34.00	2.83
	RLA027	300.00	25	23				24.00	1.41
	-	10.00	19	20				19.50	0.71
	-	30.00	17	7				12.00	7.07
	-	50.00	14	15				14.50	0.71
	-	100.00	16	19				17.50	2.12
	-	300.00	17	15				16.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIA/PLATE : 500UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

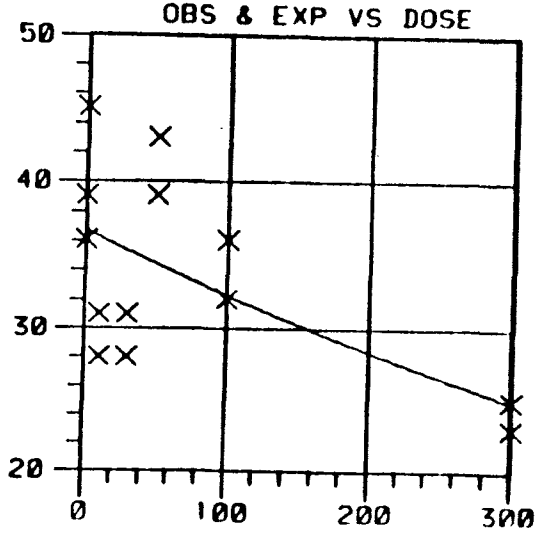
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	45	39	36	40.00	4.58
.50*	UGS	668	714	737	706.33	35.13
10.00	UGS	31	28		29.50	2.12
30.00	UGS	31	28		29.50	2.12
50.00	UGS	39	43		41.00	2.83
100.00	UGS	36	32		34.00	2.83
300.00	UGS	25	23		24.00	1.41

	B(0)	B(1)	B(2)	B(3)
ESTS.	36.675*********		.0000	.00129

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	1.87	7	.9667	-35.6165
ADEQUACY	8.19	2	.0166	-39.7126
TOXICITY	6.49	1	.0108	-42.9579
MUTAGENICITY	.01	2	.9950	-39.7126

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = .018
95% CONF. LIMITS = (-.215, .251)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/20/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	8	12	16	12.00	4.00
3.00*	UGS	492	504	551	515.67	31.18
10.00	UGS	19	20		19.50	.71
30.00	UGS	17	7		12.00	7.07
50.00	UGS	14	15		14.50	.71
100.00	UGS	16	19		17.50	2.12
300.00	UGS	17	15		16.00	1.41

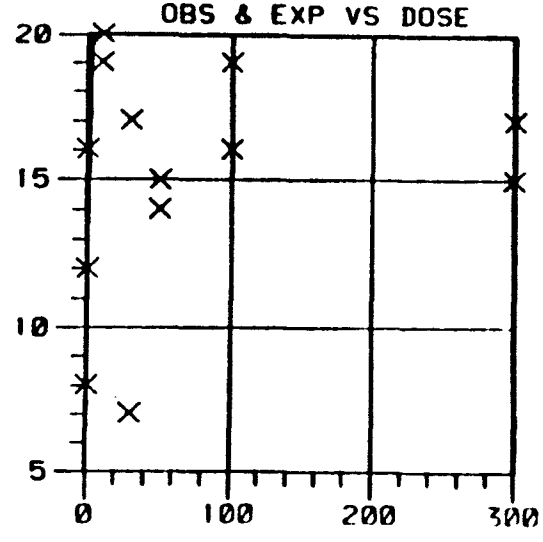
	B(0)	B(1)	B(2)
ESTS.	12.000	2.0748	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	7.28	7	.4008	-33.0852
ADEQUACY	13.07	3	.0045	-39.6227
MUTAGENICITY	.01	2	.9950	-36.4062

AVERAGE SLOPE (NONLIN. MODEL) = .080

AVERAGE SLOPE (LINEAR REGR.) = .031
95% CONF. LIMITS = (-.043, .105)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW

RESEARCH LAB: GBBA ON 03/30/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA98

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	5.00	300	312	315			309.00	7.94
2-AA	RLA026	0.50	875	837	853			855.00	19.08
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	60	41	48			49.67	9.61
	-	100.000	28	33	30			30.33	2.52
BMGS-84-0002									
	RLA026	1.00	57	51				54.00	4.24
	RLA026	5.00	55	50				52.50	3.54
	RLA026	10.00	45	52				48.50	4.95
	RLA026	30.00	55	57				56.00	1.41
	RLA026	50.00	67	54				60.50	9.19
	RLA026	100.00	61	45				53.00	11.31
	RLA026	300.00	48	51				49.50	2.12
	RLA026	500.00	48	43				45.50	3.54
	RLA026	1000.00	29	40				34.50	7.78
	-	1.00	29	26				27.50	2.12
	-	5.00	30	30				30.00	0.00
	-	10.00	48	33				40.50	10.61
	-	30.00	36	18				27.00	12.73
	-	50.00	21	29				25.00	5.66
	-	100.00	29	20				24.50	6.36
	-	300.00	29	30				29.50	0.71
	-	500.00	37	28				32.50	6.36
	-	1000.00	32	23				27.50	6.36

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-V : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS :-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	60	41	48	49.67	9.61
.50*	UGS	875	837	853	855.00	19.08
1.00	UGS	57	51		54.00	4.24
5.00	UGS	55	50		52.50	3.54
10.00	UGS	45	52		48.50	4.95
30.00	UGS	55	57		56.00	1.41
50.00	UGS	67	54		60.50	9.19
100.00	UGS	61	45		53.00	11.31
300.00	UGS	48	51		49.50	2.12

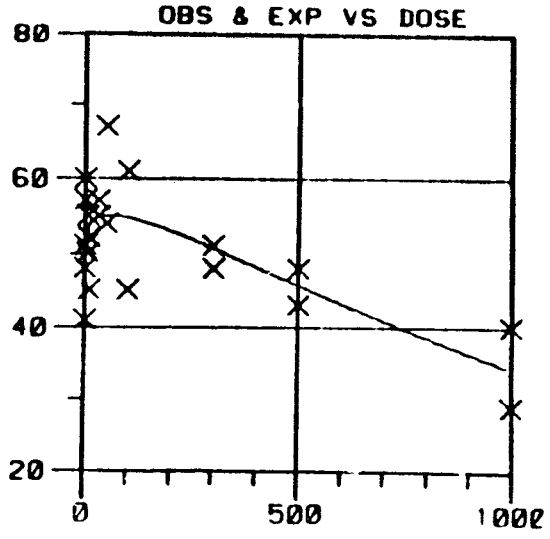
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	49.708	.8167	.2968	.00068

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	10.76	11	.4635	-65.6976
ADEQUACY	2.54	6	.8640	-66.9672
TOXICITY	19.48	1	.0000	-76.7056
MUTAGENICITY	2.39	2	.3020	-68.1645

AVERAGE SLOPE (NONLIN. MODEL) = .145
95% CONF. LIMITS = (.036, .575)

AVERAGE SLOPE (LINEAR REGR.) = .188
95% CONF. LIMITS = (.006, .370)



STATISTICAL ANALYSIS: MUTAGENICITY OF
C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	28	33	30	30.33	2.52
3.00*	UGS	300	312	315	309.00	7.94
1.00	UGS	29	26		27.50	2.12
5.00	UGS	30	30		30.00	.00
10.00	UGS	48	33		40.50	10.61
30.00	UGS	36	18		27.00	12.73
50.00	UGS	21	29		25.00	5.66
100.00	UGS	29	20		24.50	6.36
300.00	UGS	29	30		29.50	.71

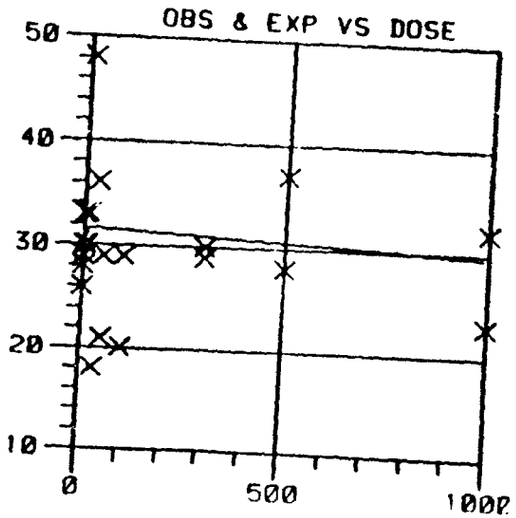
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	30.346	.2573	.0000	.00007

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	15.03	11	.1812	-62.2454
ADEQUACY	14.21	6	.0274	-69.3489
TOXICITY	2.15	1	.1424	-70.4251
MUTAGENICITY	.01	2	.9950	-68.3276

AVERAGE SLOPE (NONLIN. MODEL) = .003
95% CONF. LIMITS = (.000, .4736, .7261)

AVERAGE SLOPE (LINEAR REGR.) = .003
95% CONF. LIMITS = (-.017, .022)



MUTAGENICITY TESTING OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE YELLOW
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA98

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	250	270	255			258.33	10.41
2-AA	RLA026	0.50	740	825	817			794.00	46.94
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	42	31	43			38.67	6.66
	-	100.000	23	29	20			24.00	4.58
BMGS-84-0002									
	RLA026	1.00	45	45				45.00	0.00
	RLA026	5.00	45	54				49.50	6.36
	RLA026	10.00	40	56				48.00	11.31
	RLA026	30.00	62	69				65.50	4.95
	RLA026	50.00	49	51				50.00	1.41
	RLA026	100.00	49	40				44.50	6.36
	RLA026	300.00	56	52				54.00	2.83
	-	1.00	31	20				25.50	7.78
	-	5.00	33	25				29.00	5.66
	-	10.00	27	21				24.00	4.24
	-	30.00	28	31				29.50	2.12
	-	50.00	24	26				27.00	1.41
	-	100.00	17	30				23.50	9.19
	-	300.00	20	35				27.50	10.61

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY CHK : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=TOXIC
TNTC=TOO NUMEROUS TO COUNT
NATC=NOT ABLE TO COUNT

G-GGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	42	31	43	38.67	6.66
.50*	UGS	740	825	817	794.00	46.94
1.00	UGS	45	45		45.00	.00
5.00	UGS	45	54		49.50	6.36
10.00	UGS	40	56		48.00	11.31
30.00	UGS	62	69		65.50	4.95
50.00	UGS	49	51		50.00	1.41
100.00	UGS	49	40		44.50	6.36
300.00	UGS	56	52		54.00	2.83

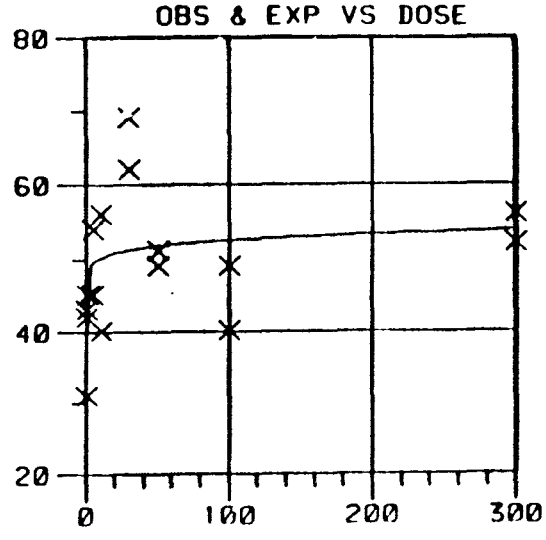
	B(0)	B(1)	B(2)
ESTS.	38.591	2.2519	.0817

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	7.25	9	.6111	-52.2272
ADEQUACY	10.63	5	.0593	-57.5407
MUTAGENICITY	9.05	2	.0108	-62.0658

AVERAGE SLOPE (NONLIN. MODEL) = .051
95% CONF. LIMITS = (.020, .126)

AVERAGE SLOPE (LINEAR REGR.) = .023
95% CONF. LIMITS = (-.024, .070)



STATISTICAL ANALYSIS: MUTAGENICITY OF C.I. SOLVENT YELLOW NO. 33 IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0002 LAB: GBBA ACTIVATION: -
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	23	29	20	24.00	4.58
3.00*	UGS	250	270	255	258.33	10.41
1.00	UGS	31	20		25.50	7.78
5.00	UGS	33	25		29.00	5.66
10.00	UGS	27	21		24.00	4.24
30.00	UGS	28	31		29.50	2.12
50.00	UGS	26	28		27.00	1.41
100.00	UGS	17	30		23.50	9.19
300.00	UGS	20	35		27.50	10.61

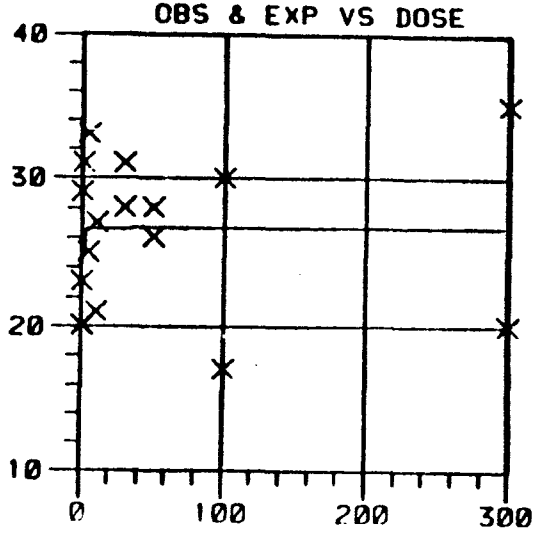
	B(0)	B(1)	B(2)
ESTS.	24.000	.9060	.0125

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	13.89	9	.1263	-50.2357
ADEQUACY	2.47	5	.7808	-51.4714
MUTAGENICITY	.64	2	.7257	-51.7920

AVERAGE SLOPE (NONLIN. MODEL) = .086
95% CONF. LIMITS = (.004, 1.836)

AVERAGE SLOPE (LINEAR REGR.) = .142
95% CONF. LIMITS = (-.117, .401)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW

RESEARCH LAB: GBBA ON 03/30/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1CO

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1179	1205	1180			1188.00	14.73
2-AA	RLA026	0.50	369	360	298			340.33	36.69
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	105	101	103			103.00	2.00
	-	100.000	103	134	97			111.33	19.86
BMGS-84-0003									
	RLA026	1.00	112	104				108.00	5.66
	RLA026	5.00	138	134				136.00	2.83
	RLA026	10.00	111	112				111.50	0.71
	RLA026	30.00	153	144				148.50	6.36
	RLA026	50.00	149	143				146.00	4.24
	RLA026	100.00	131	142				136.50	7.78
	RLA026	300.00	127	122				124.50	3.54
	RLA026	500.00	113	106				109.50	4.95
	RLA026	1000.00	101	117				109.00	11.31
	-	1.00	126	88				107.00	26.87
	-	5.00	101	127				114.00	18.38
	-	10.00	98	113				105.50	10.61
	-	30.00	102	133				117.50	21.92
	-	50.00	127	117				122.00	7.07
	-	100.00	125	109				117.00	11.31
	-	300.00	98	120				109.00	15.56
	-	500.00	113	104				108.50	6.36
	-	1000.00	88					88.00	0.00

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	105	101	103	103.00	2.00
.50*	UGS	363	360	298	340.33	36.69
1.00	UGS	112	104		108.00	5.66
5.00	UGS	138	134		136.00	2.83
10.00	UGS	111	112		111.50	.71
30.00	UGS	153	144		148.50	6.36
50.00	UGS	149	143		146.00	4.24
100.00	UGS	131	142		136.50	7.78
300.00	UGS	127	122		124.50	3.54

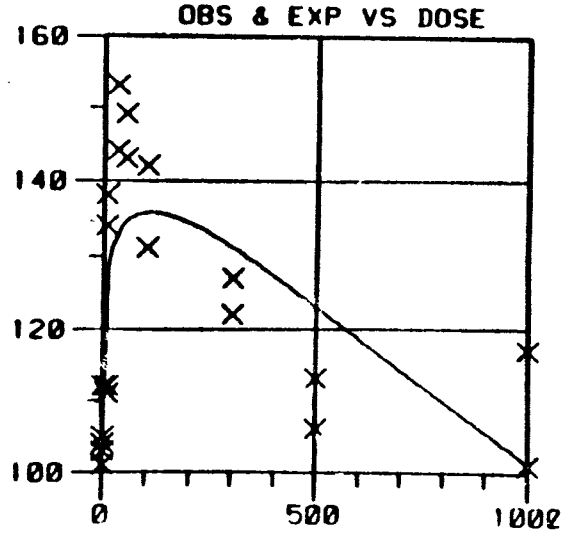
MORE THAN 8 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	101.857	2.8822	.1777	.00047

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	2.77	11	.9934	-71.0675
ADEQUACY	19.09	6	.0040	-80.6108
TOXICITY	16.01	1	.0001	-88.6153
MUTAGENICITY	22.94	2	.0000	-92.0788

AVERAGE SLOPE (NONLIN. MODEL) = 1.089
95% CONF. LIMITS = (.198, 6.002)

AVERAGE SLOPE (LINEAR REGR.) = 1.326
95% CONF. LIMITS = (.663, 1.989)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	103	134	97	111.33	19.86
3.00*	UGS	1179	1205	1180	1188.00	14.73
1.00	UGS	126	88		107.00	26.87
5.00	UGS	101	127		114.00	18.38
10.00	UGS	98	113		105.50	10.61
30.00	UGS	102	133		117.50	21.92
50.00	UGS	127	117		122.00	7.07
100.00	UGS	125	109		117.00	11.31
300.00	UGS	98	120		109.00	15.56

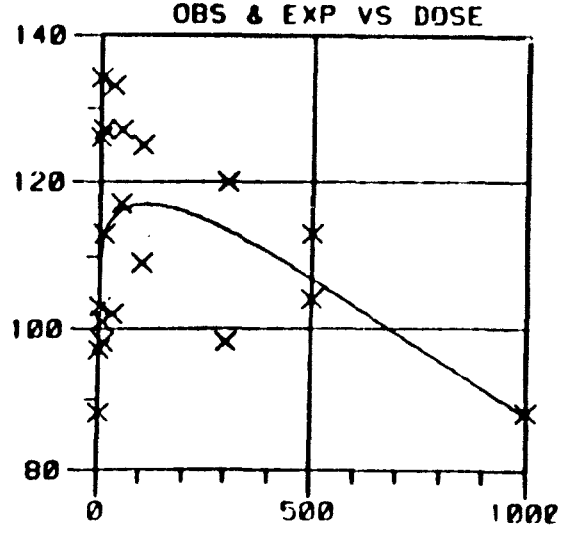
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	109.252	.3003	.5198	.00059

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	26.05	10	.0037	-78.3845
ADEQUACY	2.55	6	.8629	-79.6594
TOXICITY	7.80	1	.0052	-83.5003
MUTAGENICITY	2.82	2	.2435	-81.0718

AVERAGE SLOPE (NONLIN. MODEL) = .264
95% CONF. LIMITS = (.049, 1.425)

AVERAGE SLOPE (LINEAR REGR.) = .229
95% CONF. LIMITS = (-.715, 1.173)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA100

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1253	1350	1320			1307.67	49.66
2-AA	RLA026	0.50	953	812	842			869.00	74.28
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	115	125	114			118.00	6.08
	-	100.000	109	102	110			107.00	4.36
EMGS-84-0003									
	RLA026	1.00	108	127				117.50	13.44
	RLA026	5.00	150	113				131.50	26.16
	RLA026	10.00	152	133				142.50	13.44
	RLA026	30.00	180	175				177.50	3.54
	RLA026	50.00	125	142				133.50	12.02
	RLA026	100.00	152	134				143.00	12.73
	RLA026	300.00	144	160				152.00	11.31
	-	1.00	115	156				135.50	28.99
	-	5.00	114	137				125.50	16.26
	-	10.00	98	132				115.00	24.04
	-	30.00	115	139				127.00	16.97
	-	50.00	132	110				121.00	15.56
	-	100.00	151	102				126.50	34.65
	-	300.00	116	118				117.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-v : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50CUGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PP
N-NGS P-PP
M-MGS B-PP
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

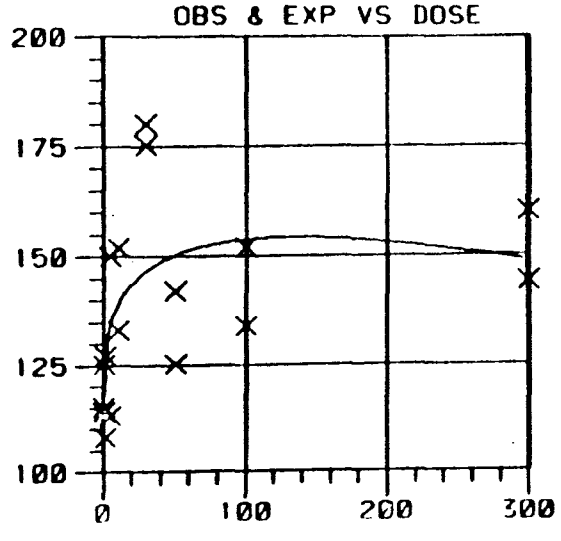
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	115	125	114	118.00	6.08
.50*	UGS	953	812	842	869.00	74.28
1.00	UGS	108	127		117.50	13.44
5.00	UGS	150	113		131.50	26.16
10.00	UGS	152	133		142.50	13.44
30.00	UGS	180	175		177.50	3.54
50.00	UGS	125	142		133.50	12.02
100.00	UGS	152	134		143.00	12.73
300.00	UGS	144	160		152.00	11.31

	B(0)	B(1)	B(2)	B(3)
ESTS.	116.104	2.4457	.3172	.00075

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	11.76	9	.2270	-63.3202
ADEQUACY	19.74	4	.0006	-73.1919
TOXICITY	2.00	1	.1576	-74.1904
MUTAGENICITY	21.14	2	.0000	-83.7619

AVERAGE SLOPE (NONLIN. MODEL) = 1.131
95% CONF. LIMITS = (.569, 2.248)

AVERAGE SLOPE (LINEAR REGR.) = 2.005
95% CONF. LIMITS = (1.360, 2.650)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

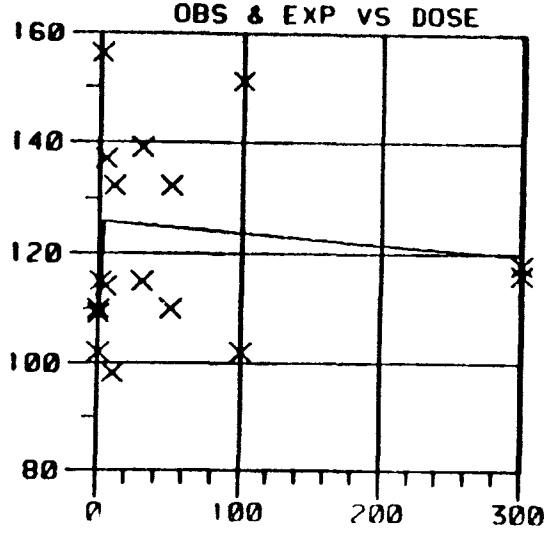
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	109	102	110	107.00	4.36
3.00*	UGS	1253	1350	1320	1307.67	49.66
1.00	UGS	115	156		135.50	28.99
5.00	UGS	114	137		125.50	16.26
10.00	UGS	98	132		115.00	24.04
30.00	UGS	115	139		127.00	16.97
50.00	UGS	132	110		121.00	15.56
100.00	UGS	151	102		126.50	34.65
300.00	UGS	116	118		117.00	1.41

	B(0)	B(1)	B(2)	B(3)
ESTS.	107.000	2.9337	.0000	.00017

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	27.47	9	.0012	-70.1066
ADEQUACY	3.82	4	.4312	-72.0157
TOXICITY	.88	1	.3487	-72.4547
MUTAGENICITY	6.82	2	.0331	-75.4241

AVERAGE SLOPE (NONLIN. MODEL) = 1.880
95% CONF. LIMITS = (.284, 12.429)

AVERAGE SLOPE (LINEAR REGR.) = .079
95% CONF. LIMITS = (-3.331, 3.489)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW

RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: PLATE TEST - PREINCUBATION STRAIN: TA100

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1227	1278	1267			1257.33	26.24
2-AA	RLA026	0.50	354	307	337			332.67	23.80
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	108	117	115			113.33	4.73
	-	100.000	144	132	127			134.33	8.74
BMGS-84-0003									
	RLA026	1.00	126	146				136.00	14.14
	RLA026	5.00	141	155				148.00	9.90
	RLA026	10.00	153	145				149.00	5.66
	RLA026	30.00	167	177				172.00	7.07
	RLA026	50.00	190	159				174.50	21.92
	RLA026	100.00	173	180				176.50	4.95
	RLA026	300.00	173	144				158.50	20.51
	-	1.00	137	129				133.00	5.66
	-	5.00	130	131				130.50	0.71
	-	10.00	131	150				140.50	13.44
	-	30.00	91	119				105.00	19.80
	-	50.00	107	126				116.50	13.44
	-	100.00	113	118				115.50	3.54
	-	300.00	89	92				90.50	2.12

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-GGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

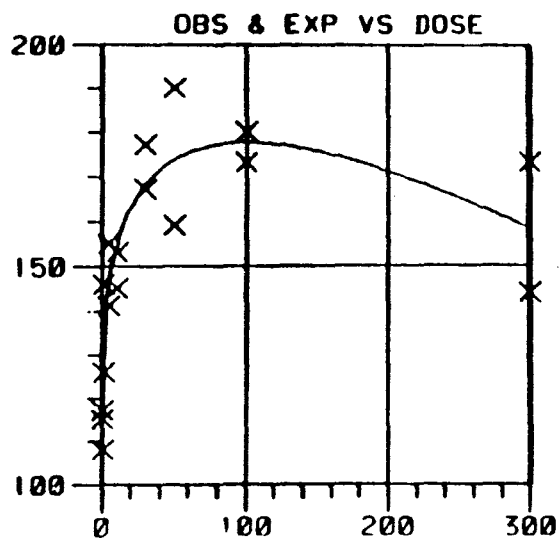
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	108	117	115	113.33	4.73
.50*	UGS	354	307	337	332.67	23.80
1.00	UGS	126	146		136.00	14.14
5.00	UGS	141	155		148.00	9.90
10.00	UGS	153	145		149.00	5.66
30.00	UGS	167	177		172.00	7.07
50.00	UGS	190	159		174.50	21.92
100.00	UGS	173	180		176.50	4.95
300.00	UGS	173	144		158.50	20.51

	B(0)	B(1)	B(2)	B(3)
ESTS	113.551	3.0270	.3241	.00145

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.58	9	.4771	-62.4701
ADEQUACY	.70	4	.9514	-62.8197
TOXICITY	8.26	1	.0040	-66.9522
MUTAGENICITY	55.31	2	.0000	-90.4723

AVERAGE SLOPE (NONLIN. MODEL) = 2.071
95% CONF. LIMITS = (1.176, 3.647)

AVERAGE SLOPE (LINEAR REGR.) = 1.628
95% CONF. LIMITS = (.903, 2.354)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA100 DATE: 04/06/84 TECHNICIAN: MJK

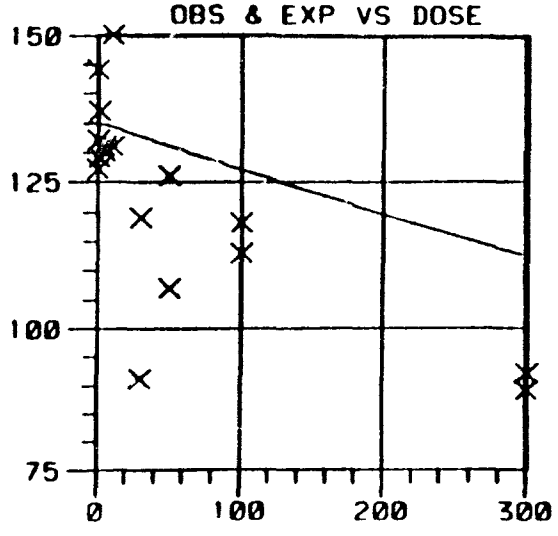
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	144	132	127	134.33	8.74
3.00*	UGS	1227	1278	1267	1257.33	26.84
1.00	UGS	137	129		133.00	5.66
5.00	UGS	130	131		130.50	.71
10.00	UGS	131	150		140.50	13.44
30.00	UGS	91	119		105.00	19.80
50.00	UGS	107	126		116.50	13.44
100.00	UGS	113	118		115.50	3.54
300.00	UGS	89	92		90.50	2.12

	B(0)	B(1)	B(2)	B(3)
ESTS.	134.379	-.3883	.0000	.00061

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.11	9	.5235	-60.3959
ADEQUACY	27.91	4	.0000	-74.3498
TOXICITY	11.82	1	.0006	-80.2595
MUTAGENICITY	.01	2	.9950	-65.5337

AVERAGE SLOPE (NONLIN. MODEL) = .068
95% CONF. LIMITS = (.000,********)

AVERAGE SLOPE (LINEAR REGR.) = .544
95% CONF. LIMITS = (-.794, 1.883)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLA027	30.00	1111	964	1069			1034.67	67.2
	-	0.50	349	335	362			355.33	24.1
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00u	108	109	100			105.67	4.9
	-	100.00u	57	40	42			46.33	9.2
BMGS-84-0003									
	RLA027	10.00	103	87				95.00	11.31
	RLA027	30.00	177	177				177.00	0.00
	RLA027	50.00	197	200				198.50	2.12
	RLA027	100.00	221	202				211.50	13.44
	RLA027	300.00	136	128				132.00	5.66
	-	10.00	99	111				105.00	8.49
	-	30.00	111	127				119.00	11.31
	-	50.00	96	86				91.00	7.07
	-	100.00	100	161				130.50	43.13
	-	300.00	138	131				134.50	4.95

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 50CUGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: 6B8A ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

*RLAC27

POSITIVE CONTROL USED WAS DANTHRON.

-

POSITIVE CONTROL USED WAS MITOMYCIN C.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/01/84 TECHNICIAN: MJK

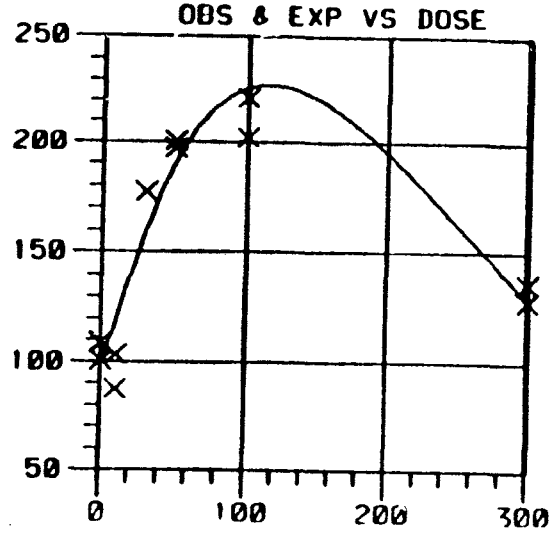
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	108	109	100	105.67	4.93
30.00*	UGS	1111	984	1009	1034.67	67.28
10.00	UGS	103	87		95.00	11.31
30.00	UGS	177	177		177.00	.00
50.00	UGS	197	200		198.50	2.12
100.00	UGS	221	202		211.50	13.44
300.00	UGS	136	128		132.00	5.66

	B(0)	B(1)	B(2)	B(3)
ESTS.	99.494	.7251	1.1552	.00838

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	2.93	7	.8917	-45.6625
ADEQUACY	17.78	2	.0001	-54.5501
TOXICITY	96.48	1	.0000	-102.7911
MUTAGENICITY	163.70	2	.0000	-136.3977

AVERAGE SLOPE (NONLIN. MODEL) = 4.220
95% CONF. LIMITS = (2.928, 6.083)

AVERAGE SLOPE (LINEAR REGR.) = 1.197
95% CONF. LIMITS = (.755, 1.638)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	57	40	42	46.33	9.29
.50*	UGS	349	335	382	355.33	24.13
10.00	UGS	99	111		105.00	8.49
30.00	UGS	111	127		119.00	11.31
50.00	UGS	96	86		91.00	7.07
100.00	UGS	100	161		130.50	43.13
300.00	UGS	138	131		134.50	4.95

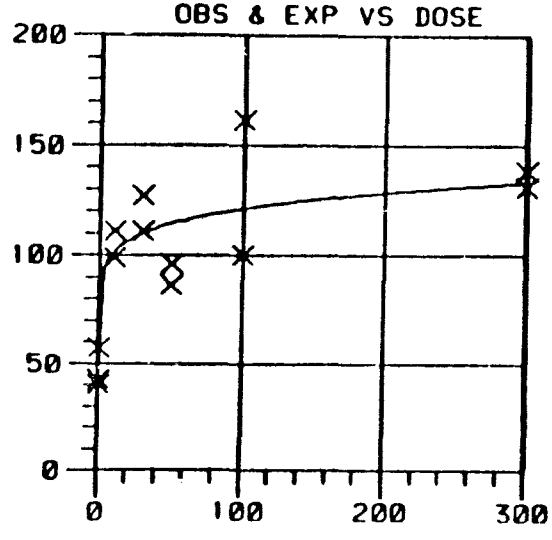
	B(0)	B(1)	B(2)
ESTS.	46.385	3.6986	.1335

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	20.48		.0046	-51.6254
ADEQUACY	13.54	3	.0036	-58.3937
MUTAGENICITY	141.98	2	.0000	-129.3820

AVERAGE SLOPE (NONLIN. MODEL) = .747
95% CONF. LIMITS = (.666, .838)

AVERAGE SLOPE (LINEAR REGR.) = .639
95% CONF. LIMITS = (.145, 1.133)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW

RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLA027	30.00	1253	1203	1096			1184.00	80.21
	-	0.50	1451	1509	1567			1489.00	32.92
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	269	325	302			298.67	28.15
	-	100.00U	215	213	203			210.33	6.43
BMGS-84-0003									
	RLA027	10.00	478	407				442.50	50.20
	RLA027	30.00	575	551				563.00	16.97
	RLA027	50.00	642	592				617.00	35.36
	RLA027	100.00	656	589				622.50	47.38
	RLA027	300.00	428	455				441.50	19.09
	-	10.00	400	503				451.50	72.83
	-	30.00	456	441				448.50	10.61
	-	50.00	468	429				448.50	27.58
	-	100.00	462	427				444.50	24.75
	-	300.00	447	424				435.50	16.26

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 300UGS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: G88A ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

*RLAC27

POSITIVE CONTROL USED WAS DANTHRON.

-

POSITIVE CONTROL USED WAS MITOMYCIN C.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: CBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/05/84 TECHNICIAN: MJK

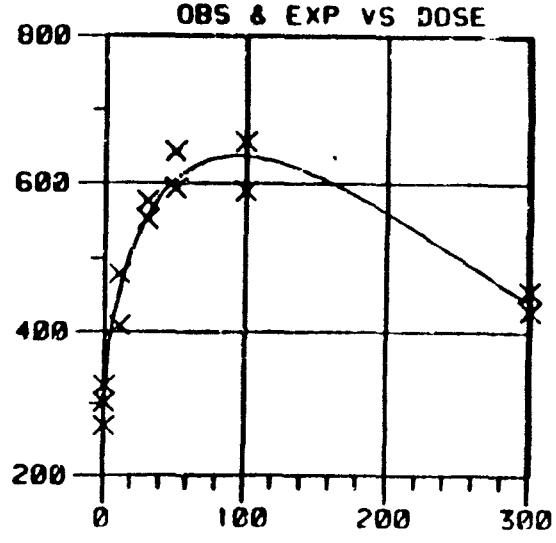
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	269	325	302	298.67	28.15
30.00*	UGS	1253	1203	1096	1184.00	80.21
10.00	UGS	478	407		442.50	50.20
30.00	UGS	575	551		563.00	16.97
50.00	UGS	642	592		617.00	35.36
100.00	UGS	656	589		622.50	47.38
300.00	UGS	428	455		441.50	19.09

	B(0)	B(1)	B(2)	B(3)
ESTS.	297.756	3.7944	.5934	.00433

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	17.97	7	.0121	-60.8696
ADEQUACY	1.77	2	.4132	-61.7534
TOXICITY	120.57	1	.0000	-122.0388
MUTAGENICITY	433.32	2	.0000	-278.4109

AVERAGE SLOPE (NONLIN. MODEL) = 9.059
95% CONF. LIMITS = (8.120, 10.097)

AVERAGE SLOPE (LINEAR REGR.) = 6.326
95% CONF. LIMITS = (4.550, 8.103)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/05/84 TECHNICIAN: MJK

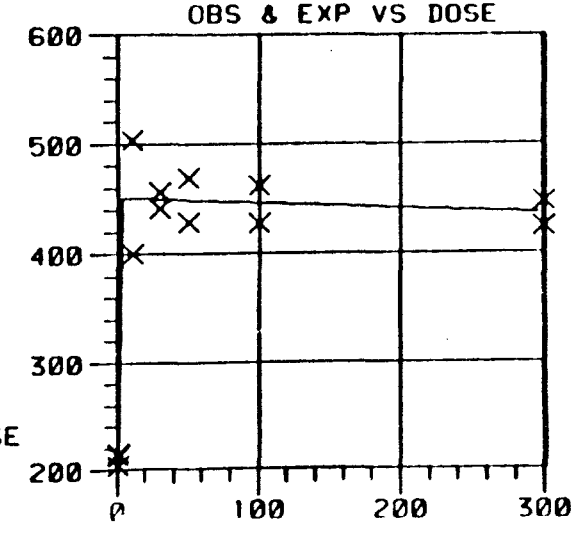
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	215	213	203	210.33	6.43
.50*	UGS	1451	1509	1507	1489.00	32.02
10.00	UGS	400	503		451.50	72.83
30.00	UGS	456	441		448.50	10.61
50.00	UGS	468	429		448.50	27.58
100.00	UGS	462	427		444.50	24.75
300.00	UGS	447	424		435.50	16.26

	B(0)	B(1)	B(2)	B(3)
ESTS.	210.329	5.4833	.0000	.00011

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	16.07	7	.0245	-58.5117
ADEQUACY	.02	2	.9906	-58.5211
TOXICITY	3.87	1	.0492	-60.4549
MUTAGENICITY	385.47	2	0000	-251.2576

AVERAGE SLOPE (NONLIN. MODEL) = 4.813
95% CONF. LIMITS = (3.739, 6.194)

AVERAGE SLOPE (LINEAR REGR.) = 4.185
95% CONF. LIMITS = (.980, 7.390)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/15/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA102

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
OTHER POS	RLA027	0.50	1252	1377	1392	1370		1347.75	64.49
	-	30.00	1380	1320	1363			1361.00	35.54
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	264	264	266			264.67	1.15
	-	100.00U	202	188	213			201.00	12.53
BMGS-84-3003									
	RLA027	1.00	279	239				259.00	28.28
	RLA027	5.00	329	334				331.50	3.54
	RLA027	10.00	364	392				378.00	19.80
	RLA027	30.00	538	532				535.00	4.24
	RLA027	50.00	626	638				632.00	8.49
	RLA027	100.00	656	643				649.50	9.19
	RLA027	300.00	639	619				629.00	14.14
	-	1.00	211	217				214.00	4.24
	-	5.00	313	304				308.50	6.36
	-	10.00	341	342				341.50	0.71
	-	30.00	404	426				415.00	15.56
	-	50.00	475	407				441.00	48.08
	-	100.00	441	413				427.00	19.80
	-	300.00	368	400				384.00	22.63

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 5000GS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

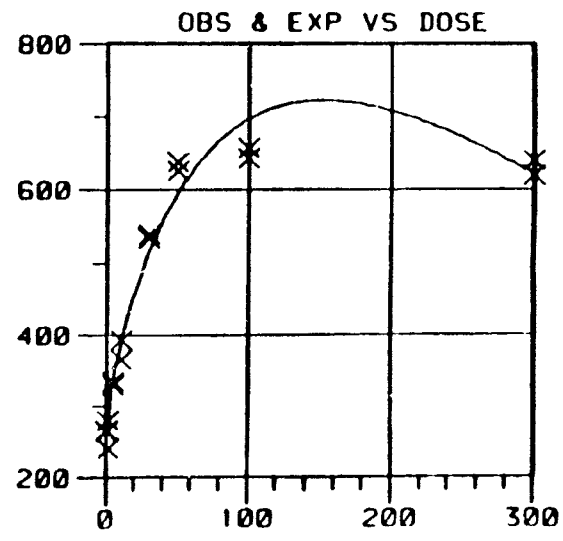
DOSE	UNITS	PLATE COUNTS				MEAN	S.D.
.00	UGS	264	264	266		264.67	1.16
.50*	UGS	1252	1377	1392	1370	1347.75	64.49
1.00	UGS	279	239			259.00	28.28
5.00	UGS	329	334			331.50	3.54
10.00	UGS	364	392			378.00	19.80
30.00	UGS	538	532			535.00	4.24
50.00	UGS	626	638			632.00	8.49
100.00	UGS	656	643			649.50	9.19
300.00	UGS	639	619			629.00	14.14

	B(0)	B(1)	B(2)	B(3)
ESTS.	252.524	3.3777	.7060	.00372

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.77	9	.8539	-69.3218
ADEQUACY	18.35	4	.0011	-78.4985
TOXICITY	131.22	1	.0000	-144.1083
MUTAGENICITY	1013.78	2	.0000	-585.3907

AVERAGE SLOPE (NONLIN. MODEL) = 7.567
95% CONF. LIMITS = (6.535, 8.762)

AVERAGE SLOPE (LINEAR REGR.) = 4.181
95% CONF. LIMITS = (3.031, 5.332)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA102 DATE: 06/15/84 TECHNICIAN: MJK

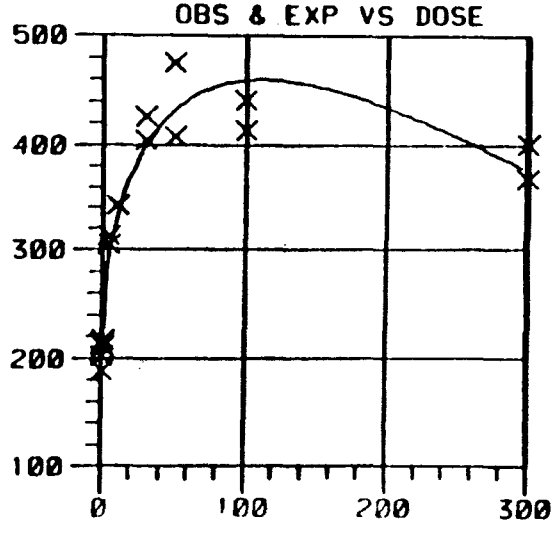
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	202	188	213	201.00	12.53
30.00*	UGS	1380	1320	1383	1361.00	35.54
1.00	UGS	211	217		214.00	4.24
5.00	UGS	313	304		308.50	6.36
10.00	UGS	341	342		341.50	.71
30.00	UGS	404	426		415.00	15.56
50.00	UGS	475	407		441.00	48.08
100.00	UGS	441	413		427.00	19.80
300.00	UGS	368	400		384.00	22.63

	B(0)	B(1)	B(2)	B(3)
ESTS.	194.489	3.9460	.4507	.00279

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	9.86	9	.3623	-69.5632
ADEQUACY	17.08	4	.0019	-78.1015
TOXICITY	79.42	1	.0000	-117.8105
MUTAGENICITY	442.87	2	.0000	-299.5369

AVERAGE SLOPE (NONLIN. MODEL) = 6.032
95% CONF. LIMITS = (5.524, 6.587)

AVERAGE SLOPE (LINEAR REGR.) = 4.656
95% CONF. LIMITS = (3.219, 6.093)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-AA	RLA027	3.00	2393	2440	2467			2433.33	37.45
OTHER POS	-	17.00	765	851	777			797.67	46.58
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	312	311	340			321.00	16.46
	-	100.000	262	323	271			285.33	32.93
BMGS-84-0003									
	RLA027	10.00	460	515				487.50	38.89
	RLA027	30.00	476	516				496.00	28.28
	RLA027	50.00	485	412				448.50	51.62
	RLA027	100.00	273	301				287.00	19.80
	RLA027	300.00	439	377				408.00	43.84
	-	10.00	381	378				379.50	2.12
	-	30.00	347	341				344.00	4.24
	-	50.00	330	346				338.00	11.31
	-	100.00	359	318				338.50	28.99
	-	300.00	309	297				303.00	8.49

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/01/84 TECHNICIAN: MJK

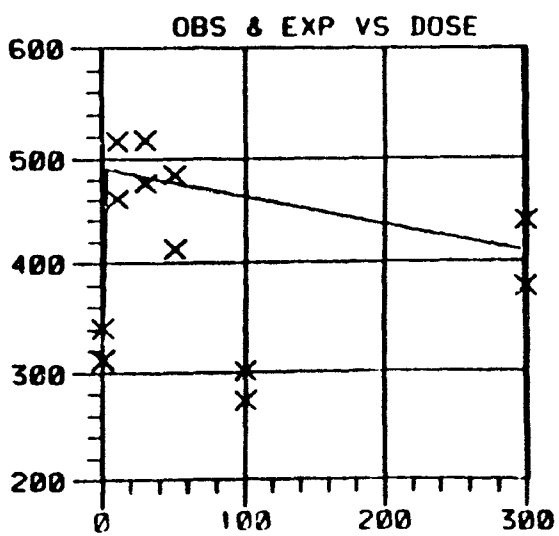
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	312	311	340	321.00	16.46
3.00*	UGS	2393	2440	2467	2433.33	37.45
10.00	UGS	460	515		487.50	38.89
30.00	UGS	476	516		496.00	28.28
50.00	UGS	485	412		448.5[illegible]	51.62
100.00	UGS	273	301		287.0[illegible]	19.80
300.00	UGS	439	377		408.00	43.84

	B(0)	B(1)	B(2)	B(3)
ESTS.	321.000	5.1383	.0000	.00061

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	18.42	7	.0102	-59.9766
ADEQUACY	158.35	2	.0000	-139.1502
TOXICITY	61.28	1	.0000	-169.7917
MUTAGENICITY	52.03	2	.0000	-165.1676

AVERAGE SLOPE (NONLIN. MODEL) = .568
95% CONF. LIMITS = (.443, .729)

AVERAGE SLOPE (LINEAR REGR.) = -.050
95% CONF. LIMITS = (-.547, .446)



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STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/01/84 TECHNICIAN: MJK

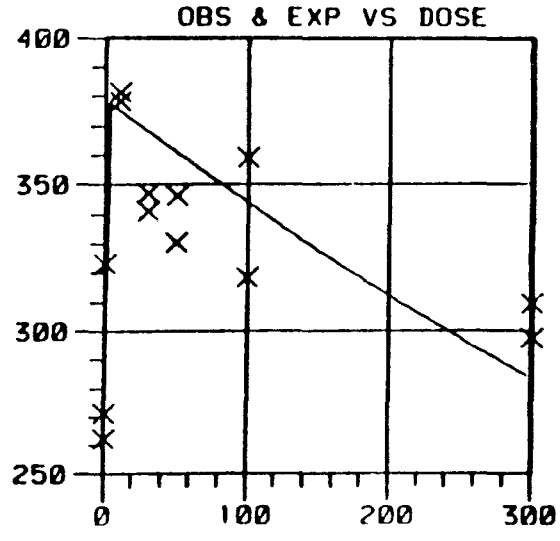
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	262	323	271	285.33	32.93
17.00*	UGS	765	851	777	797.67	46.58
10.00	UGS	381	378		379.50	2.12
30.00	UGS	347	341		344.00	4.24
50.00	UGS	330	346		338.00	11.31
100.00	UGS	359	318		338.50	28.99
300.00	UGS	309	297		303.00	8.49

	B(0)	B(1)	B(2)	B(3)
ESTS.	285.337	4.5354	.0000	.00097

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	10.76	7	.1493	-54.8752
ADEQUACY	9.07	2	.0107	-59.4089
TOXICITY	13.62	1	.0002	-66.2179
MUTAGENICITY	28.25	2	.0000	-73.5332

AVERAGE SLOPE (NONLIN. MODEL) = 1.865
95% CONF. LIMITS = (1.349, 2.5791)

AVERAGE SLOPE (LINEAR REGR.) = .721
95% CONF. LIMITS = (-.697, 2.1381)



A-140

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1C4

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-AA	RLA027	3.00	2424	2346	2444			2405.33	50.65
OTHER POS	-	50.00	1657	1786	1736			1726.33	65.04
NEG CONTROL									
DIMETHYLSULF	RLA027	100.00U	350	316	290			318.67	30.09
	-	100.00U	241	252	222			238.33	15.18
BMGS-84-0003									
	RLA027	1.00	352	334				343.00	12.73
	RLA027	5.00	424	366				395.00	41.01
	RLA027	10.00	439	477				458.00	26.87
	RLA027	30.00	526	503				514.50	16.26
	RLA027	50.00	491	540				515.50	34.65
	RLA027	100.00	447	440				443.50	4.95
	RLA027	300.00	410	402				406.00	5.66
	-	1.00	263	254				258.50	6.36
	-	5.00	258	288				273.00	21.21
	-	10.00	291	301				296.00	7.07
	-	30.00	294	290				292.00	2.83
	-	50.00	315	307				311.00	5.66
	-	100.00	293	291				292.00	1.41
	-	300.00	294	284				289.00	7.07

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY 5-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

POSITIVE CONTROL USED WAS METHYL GLYOXAL.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN: MJK

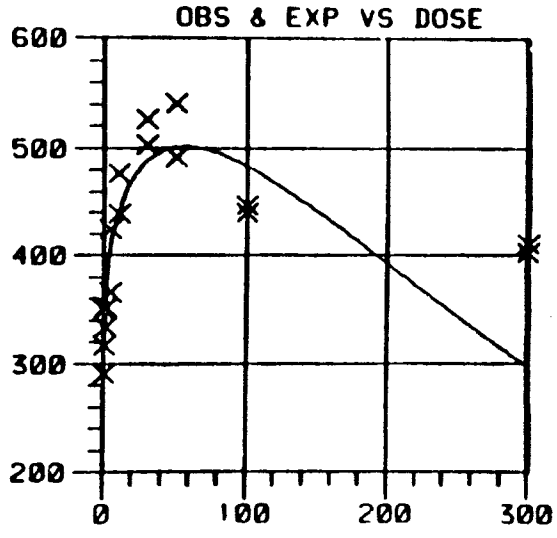
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	350	316	290	318.67	30.09
3.00*	UGS	2424	2348	2444	2405.33	50.65
1.00	UGS	352	334		343.00	12.73
5.00	UGS	424	366		395.00	41.01
10.00	UGS	439	477		458.00	26.87
30.00	UGS	526	503		514.50	16.26
50.00	UGS	491	540		515.50	34.65
100.00	UGS	447	440		443.50	4.95
300.00	UGS	410	402		406.00	5.66

	B(0)	B(1)	B(2)	B(3)
ESTS.	312.210	3.9616	.4431	.00394

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	14.97	9	.0919	-74.2715
ADEQUACY	88.65	4	.0000	-118.5981
TOXICITY	2.50	1	.1136	-119.8498
MUTAGENICITY	116.51	2	.0000	-176.8549

AVERAGE SLOPE (NONLIN. MODEL) = 7.905

AVERAGE SLOPE (LINEAR REGR.) = 6.283
95% CONF. LIMITS = (4.136, 8.430)
WARNING: 4 PARAMETER MODEL DID NOT CONVERGE



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA. ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA104

POSITIVE CONTROL USED WAS METHYL GLYOXAL.

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA104 DATE: 06/05/84 TECHNICIAN: MJK

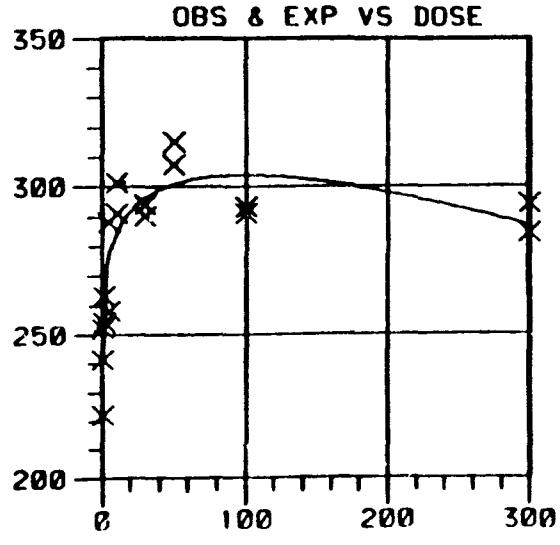
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	241	252	222	238.33	15.18
50.00*	UGS	1657	1786	1736	1726.33	65.04
1.00	UGS	263	254		258.50	6.36
5.00	UGS	258	288		273.00	21.21
10.00	UGS	291	301		296.00	7.07
30.00	UGS	294	290		292.00	2.83
50.00	UGS	315	307		311.00	5.66
100.00	UGS	293	291		292.00	1.41
300.00	UGS	294	284		289.00	7.07

	B(0)	B(1)	B(2)	B(3)
ESTS.	237.591	3.2852	.2606	.00072

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.22	9	.8966	-65.5625
ADEQUACY	3.10	4	.5420	-67.1101
TOXICITY	4.01	1	.0452	-69.1151
MUTAGENICITY	31.25	2	.0000	-82.7328

AVERAGE SLOPE (NONLIN. MODEL) = 1.481
95% CONF. LIMITS = (.711, 3.084)

AVERAGE SLOPE (LINEAR REGR.) = 1.179
95% CONF. LIMITS = (.606, 1.753)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1535

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1021	1031	959			1003.67	39.00
2-AA	RLA027	3.00	153	164	132			149.67	16.26
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	26	24	29			26.33	2.52
	-	100.000	37	44	33			38.00	5.57
BMGS-84-0003									
	RLA027	10.00	18	13				15.50	3.54
	RLA027	30.00	14	16				15.00	1.41
	RLA027	50.00	27	19				23.00	5.66
	RLA027	100.00	19	17				18.00	1.41
	RLA027	300.00	22	20				21.00	1.41
	-	10.00	29	42				35.50	9.19
	-	30.00	44	40				42.00	2.83
	-	50.00	36	32				34.00	2.83
	-	100.00	36	42				39.00	4.24
	-	300.00	42					42.00	0.00

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-PM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/01/84 TECHNICIAN: MJK

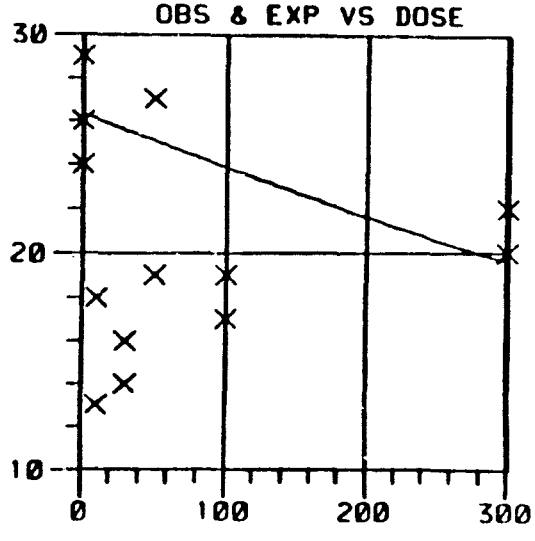
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	26	24	29	26.33	2.52
3.00*	UGS	153	164	132	149.67	16.26
10.00	UGS	18	13		15.50	3.54
30.00	UGS	14	16		15.00	1.41
50.00	UGS	27	19		23.00	5.66
100.00	UGS	19	17		18.00	1.41
300.00	UGS	22	20		21.00	1.41

	B(0)	B(1)	B(2)	B(3)
ESTS.	26.272	-2.7822	.0002	.00099

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.02	7	.8833	-32.9037
ADEQUACY	24.02	2	.0000	-44.9131
TOXICITY	7.01	1	.0081	-48.4184
MUTAGENICITY	.01	2	.9950	-38.7526

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000,********)

AVERAGE SLOPE (LINEAR REGR.) = -.001
95% CONF. LIMITS = (-.030, .027)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	37	44	33	38.00	5.57
3.00*	UGS	1021	1031	959	1003.67	39.00
10.00	UGS	29	42		35.50	9.19
30.00	UGS	44	40		42.00	2.83
50.00	UGS	36	32		34.00	2.83
100.00	UGS	36	42		39.00	4.24
300.00	UGS	42			42.00	.00

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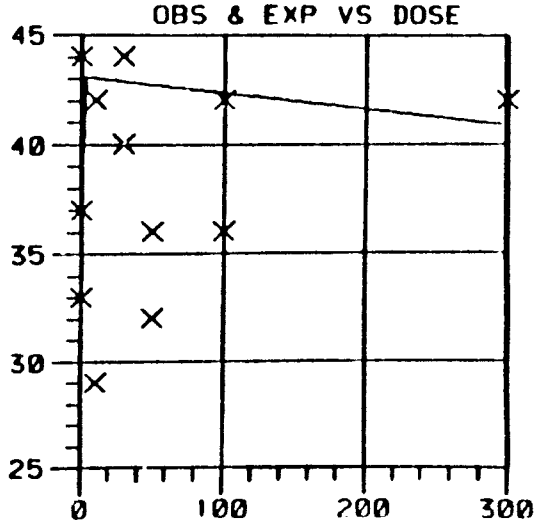
	B(0)	B(1)	B(2)	B(3)
ESTS.	38.000	1.6287	.0000	.00018

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.90	6	.5568	-35.2919
ADEQUACY	7.24	2	.0268	-38.9100
TOXICITY	.96	1	.3263	-39.3916
MUTAGENICITY	.01	2	.9950	-36.5480

AVERAGE SLOPE (NONLIN. MODEL) = .051
95% CONF. LIMITS = (.000, 14.973)

AVERAGE SLOPE (LINEAR REGR.) = .007
95% CONF. LIMITS = (-.084, .098)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1535

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
NAAZIDE	-	3.00	1068	1057	1018			1047.67	26.27
2-AA	RLA027	3.00	154	154	157			155.00	1.73
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	32	33	18			27.67	8.39
	-	100.000	55	50	40			48.33	7.64
EMGS-84-0003									
	RLA027	10.00	14	18				16.00	2.83
	RLA027	30.00	9	17				13.00	5.66
	RLA027	50.00	24	28				26.00	2.83
	RLA027	100.00	19	20				19.50	0.71
	RLA027	300.00	21	22				21.50	0.71
	-	10.00	42	55				48.50	9.19
	-	30.00	39	39				39.00	0.00
	-	50.00	50	37				43.50	9.19
	-	100.00	48	43				45.50	3.54
	-	300.00	51	50				50.50	0.71

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	32	33	18	27.67	8.39
3.00*	UGS	154	154	157	155.00	1.73
10.00	UGS	14	18		16.00	2.83
30.00	UGS	9	17		13.00.	5.66
50.00	UGS	24	28		26.00	2.83
100.00	UGS	19	20		19.50	.71
300.00	UGS	21	22		21.50	.71

A-149

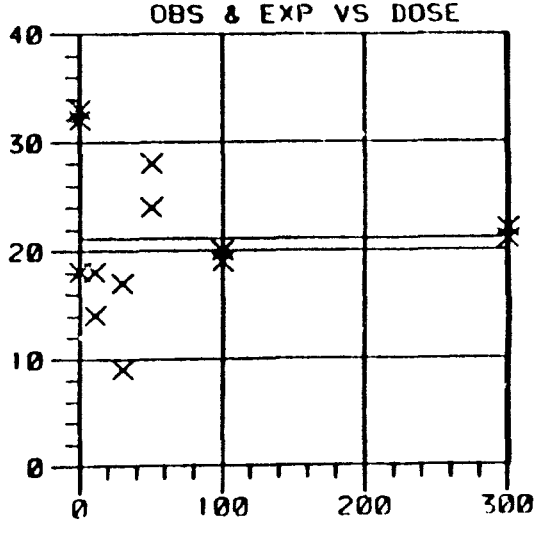
	B(0)	B(1)	B(2)
ESTS.	21.160	-320.3121	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.40	7	.2984	-35.9188
ADEQUACY	17.87	3	.0005	-44.8517
MUTAGENICITY	.00	21	.0000	-44.8517

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000, .000)

AVERAGE SLOPE (LINEAR REGR.) = -.002
95% CONF. LIMITS = (-.041, .037)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA1535 DATE: 06/05/84 TECHNICIAN: MJK

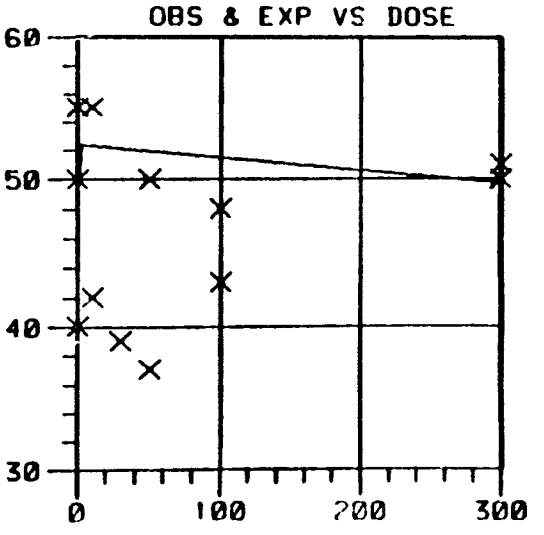
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	55	50	40	48.33	7.64
3.00*	UGS	1068	1057	1018	1047.67	26.27
10.00	UGS	42	55		48.50	9.19
30.00	UGS	39	39		39.00	.00
50.00	UGS	50	37		43.50	9.19
100.00	UGS	48	43		45.50	3.54
300.00	UGS	51	50		50.50	.71

	B(0)	B(1)	B(2)	B(3)
ESTS.	48.332	1.3938	.0000	.00017

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.38	7	.4958	-40.0330
ADEQUACY	12.11	2	.0023	-46.0889
TOXICITY	6.92	1	.0085	-49.5500
MUTAGENICITY	.01	2	.9950	-42.0575

AVERAGE SLOPE (NONLIN. MODEL) = .013
95% CONF. LIMITS = (.000, 226.752)

AVERAGE SLOPE (LINEAR REGR.) = .014
95% CONF. LIMITS = (-.021, .049)
WARNING: 3 PARAMETER MODEL DID NOT CONVERGE



A-150

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1537

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
9-AA	-	100.00	1129	1111	860			1040.00	138.86
2-AA	RLA027	5.00	304	380	343			342.33	38.00
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	17	15	27			19.67	6.43
	-	100.000	14	18	8			13.33	5.03
EMGS-34-0003									
	RLA027	10.00	18	19				18.50	0.71
	RLA027	30.00	36	33				34.50	2.12
	RLA027	50.00	39	38				38.50	0.71
	RLA027	100.00	24	22				23.00	1.41
	RLA027	300.00	21	19				20.00	1.41
	-	10.00	19	12				15.50	4.95
	-	30.00	14	17				15.50	2.12
	-	50.00	20	7				13.50	9.19
	-	100.00	26	14				20.00	8.49
	-	300.00	12	10				11.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 5.00UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: MJK

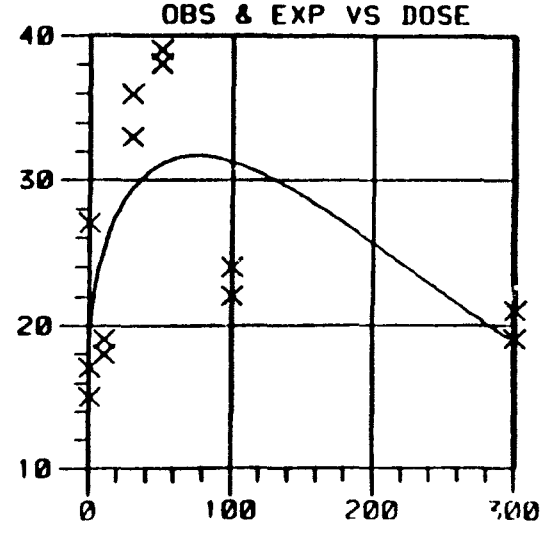
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	17	15	27	19.67	6.43
3.00*	UGS	304	380	343	342.33	38.00
10.00	UGS	18	19		18.50	.71
30.00	UGS	36	33		34.50	2.12
50.00	UGS	39	38		38.50	.71
100.00	UGS	24	22		23.00	1.41
300.00	UGS	21	19		20.00	1.41

	B(0)	B(1)	B(2)	B(3)
ESTS.	18.882	.5697	.6336	.00501

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	4.56	7	.7134	-34.8443
ADEQUACY	13.94	2	.0009	-41.8119
TOXICITY	12.85	1	.0003	-48.2346
MUTAGENICITY	13.83	2	.0010	-48.7252

AVERAGE SLOPE (NONLIN. MODEL) = .422
95% CONF. LIMITS = (.191, .929)

AVERAGE SLOPE (LINEAR REGR.) = .426
95% CONF. LIMITS = (.266, .587)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/01/84 TECHNICIAN: MJK

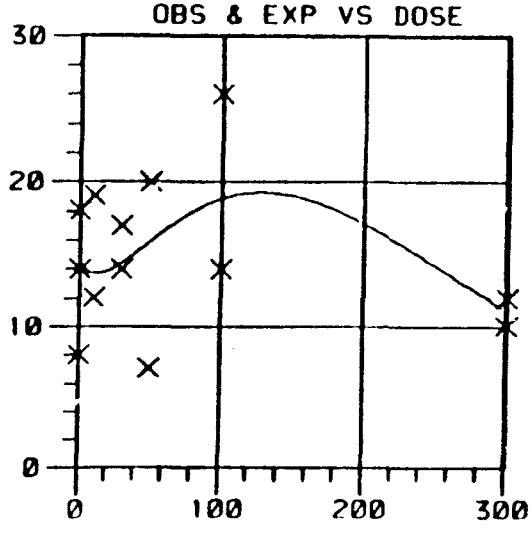
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	14	18	8	13.33	5.03
100.00*	UGS	1129	1111	880	1040.00	138.86
10.00	UGS	19	12		15.50	4.95
30.00	UGS	14	17		15.50	2.12
50.00	UGS	20	7		13.50	9.19
100.00	UGS	26	14		20.00	8.49
300.00	UGS	12	10		11.00	1.41

	B(0)	B(1)	B(2)	B(3)
ESTS.	14.348	-3.9506	1.6535	.01042

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	15.71	7	.0279	-37.2216
ADEQUACY	1.74	2	.4189	-38.0917
TOXICITY	5.16	1	.0231	-40.6723
MUTAGENICITY	3.73	2	.1552	-39.9550

AVERAGE SLOPE (NONLIN. MODEL) = .248
95% CONF. LIMITS = (.069, .887)

AVERAGE SLOPE (LINEAR REGR.) = .003
95% CONF. LIMITS = (-.168, .174)



A-153

MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1537

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
9-AA	-	100.00	1258	1335	1452			1348.33	97.68
2-AA	RLA027	5.00	561	472	408			480.33	76.84
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	20	16	17			17.67	2.08
	-	100.000	8	8	9			8.33	0.58
BMGS-84-0003									
	RLA027	10.00	28	26				27.00	1.41
	RLA027	30.00	42	36				39.00	4.24
	RLA027	50.00	30	30				30.00	0.00
	RLA027	100.00	37	31				34.00	4.24
	RLA027	300.00	19	30				24.50	7.78
	-	10.00	20	20				20.00	0.00
	-	30.00	17	19				18.00	1.41
	-	50.00	14	12				13.00	1.41
	-	100.00	13	19				16.00	4.24
	-	300.00	21	12				16.50	6.36

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T--TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-PM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: MJK

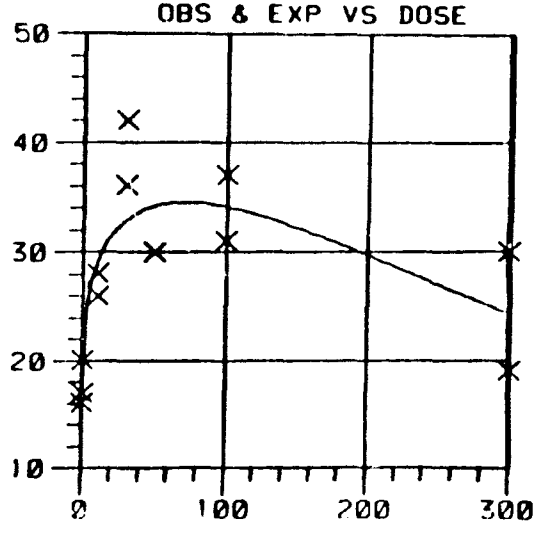
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	20	16	17	17.67	2.08
3.00*	UGS	561	472	408	480.33	76.84
10.00	UGS	28	26		27.00	1.41
30.00	UGS	42	36		39.00	4.24
50.00	UGS	30	30		30.00	.00
100.00	UGS	37	31		34.00	4.24
300.00	UGS	19	30		24.50	7.78

	B(0)	B(1)	B(2)	B(3)
ESTS.	17.613	1.6844	.3609	.00300

TEST	CHI-SQUARE	DF	P	LOCL
POISSON	4.02	7	.7769	-35.3616
ADEQUACY	3.52	2	.1723	-37.1201
TOXICITY	5.81	1	.0159	-40.0245
MUTAGENICITY	21.64	2	.0000	-47.9387

AVERAGE SLOPE (NONLIN. MODEL) = .284
95% CONF. LIMITS = (.062, 1.292)

AVERAGE SLOPE (LINEAR REGR.) = .135
95% CONF. LIMITS = (.016, .253)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA1537 DATE: 06/05/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	8	8	9	8.33	.58
100.00*	UGS	1258	1335	1452	1348.33	97.69
10.00	UGS	20	20		20.00	.00
30.00	UGS	17	19		18.00	1.41
50.00	UGS	14	12		13.00	1.41
100.00	UGS	13	19		16.00	4.24
300.00	UGS	21	12		16.50	6.36

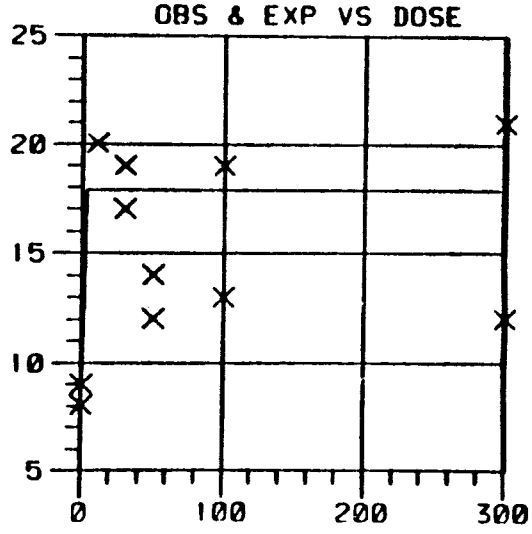
	B(0)	B(1)	B(2)
ESTS.	8.251	2.2653	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.92	7	.7884	-31.1534
ADEQUACY	4.07	3	.2542	-33.1877
MUTAGENICITY	11.62	2	.0030	-38.9962

AVERAGE SLOPE (NONLIN. MODEL) = .096
95% CONF. LIMITS = (.012, .781)

AVERAGE SLOPE (LINEAR REGR.) = .035
95% CONF. LIMITS = (-.048, .118)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: 688A ON 06/01/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	476	432	512			473.33	40.07
2-AA	RLA027	0.50	704	729	692			708.33	18.88
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	26	25	24			25.00	1.00
	-	100.000	17	13	18			16.00	2.65
BMGS-34-0003									
	RLA027	10.00	25	38				31.50	9.19
	RLA027	30.00	29	28				28.50	0.71
	RLA027	50.00	21	37				29.00	11.31
	RLA027	100.00	17	42				29.50	17.68
	RLA027	300.00	28	20				24.00	5.66
	-	10.00	26	19				22.50	4.95
	-	30.00	19	18				18.50	0.71
	-	50.00	20	17				18.50	2.12
	-	100.00	15	19				17.00	2.83
	-	300.00	21	17				19.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: MJK

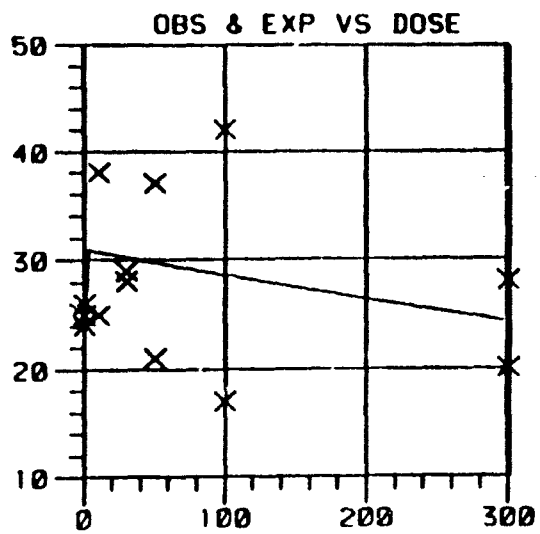
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	26	25	24	25.00	1.00
.50*	UGS	704	729	682	708.33	18.88
10.00	UGS	25	38		31.50	9.19
30.00	UGS	29	28		28.50	.71
50.00	UGS	21	37		29.00	11.31
100.00	UGS	17	42		29.50	17.68
300.00	UGS	28	20		24.00	5.66

	B(0)	B(1)	B(2)	B(3)
ESTS.	25.000	1.7852	.0000	.00080

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	19.12	7	.0078	-43.1435
ADEQUACY	.35	2	.8409	-43.3168
TOXICITY	6.48	1	.0109	-46.5550
MUTAGENICITY	2.19	2	.3347	-44.4113

AVERAGE SLOPE (NONLIN. MODEL) = .119
95% CONF. LIMITS = (.000, 48.867)

AVERAGE SLOPE (LINEAR REGR.) = .053
95% CONF. LIMITS = (-.156, .262)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/01/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	17	13	18	16.00	2.65
3.00*	UGS	476	432	512	473.33	40.07
10.00	UGS	26	19		22.50	4.95
30.00	UGS	19	18		18.50	.71
50.00	UGS	20	17		18.50	2.12
100.00	UGS	15	19		17.00	2.83
300.00	UGS	21	17		19.00	2.83

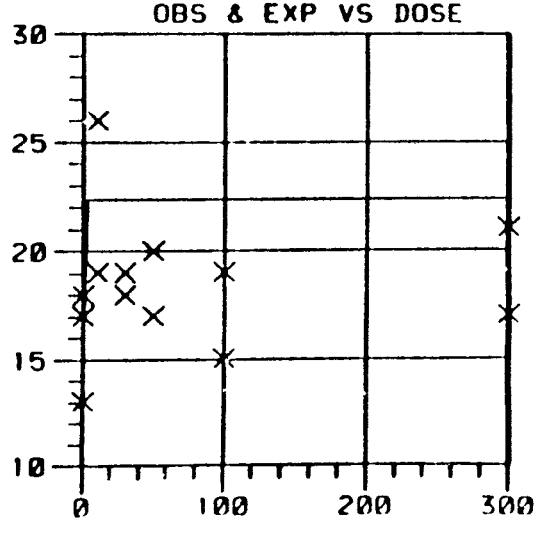
	B(0)	B(1)	B(2)
ESTS.	16.000	1.8443	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	3.13	7	.8731	-32.4292
ADEQUACY	6.60	3	.0858	-35.7287
MUTAGENICITY	.01	2	.9950	-33.9027

AVERAGE SLOPE (NONLIN. MODEL) = .126
95% CONF. LIMITS = (.030, .542)

AVERAGE SLOPE (LINEAR REGR.) = .021
95% CONF. LIMITS = (-.104, .145)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 06/05/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA1538

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	615	652	628			631.67	18.77
2-AA	RLA027	0.50	1061	1063	1052			1058.67	5.86
NEG CONTROL									
DIMETHYLSULF	RLA027	100.000	41	19	36			32.00	11.53
	-	100.000	18	13	15			15.33	2.52
GMGS-84-0003									
	RLA027	10.00	32	30				31.00	1.41
	RLA027	30.00	44	45				44.50	0.71
	RLA027	50.00	27	33				30.00	4.24
	RLA027	100.00	30	32				31.00	1.41
	RLA027	300.00	29	32				30.50	2.12
	-	10.00	14	14				14.00	0.00
	-	30.00	20	18				19.00	1.41
	-	50.00	9	16				12.50	4.95
	-	100.00	19	14				16.50	3.54
	-	300.00	9	17				13.00	5.66

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA027
STRAIN: TA1538 DATE: 06/05/84 TECHNICIAN: MJK

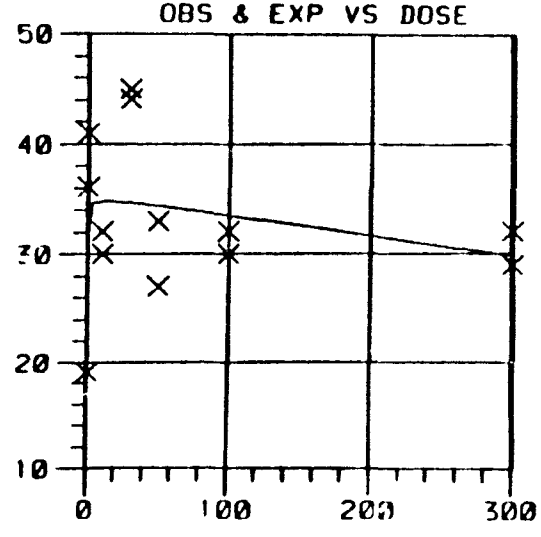
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	41	19	36	32.00	11.53
.50*	UGS	1061	1063	1052	1058.67	5.86
10.00	UGS	32	30		31.00	1.41
30.00	UGS	44	45		44.50	.71
50.00	UGS	27	33		30.00	4.24
100.00	UGS	30	32		31.00	1.41
300.00	UGS	29	32		30.50	2.12

	B(0)	B(1)	B(2)	B(3)
ESTS.	31.986	.8620	.1005	.00065

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	9.20	7	.2386	-39.5176
ADEQUACY	7.56	2	.0228	-43.2975
TOXICITY	6.87	1	.0088	-46.7338
MUTAGENICITY	.58	2	.7471	-43.5890

AVERAGE SLOPE (NONLIN. MODEL) = .070
95% CONF. LIMITS = (.001, 5.188)

AVERAGE SLOPE (LINEAR REGR.) = .044
95% CONF. LIMITS = (-.263, .352)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA1538 DATE: 06/05/84 TECHNICIAN: MJK

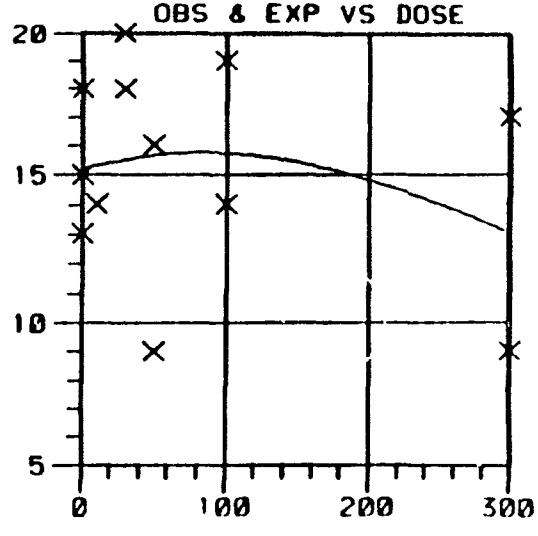
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	18	13	15	15.33	2.52
3.00*	UGS	615	652	628	631.67	18.77
10.00	UGS	14	14		14.00	.00
30.00	UGS	20	18		19.00	1.41
50 00	UGS	9	16		12.50	4.95
100.00	UGS	19	14		16.50	3.54
300.00	UGS	9	17		13.00	5.66

	B(0)	B(1)	B(2)	B(3)
ESTS.	15.205	-2.8680	1.0597	.00366

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	6.11	7	.5269	-32.5657
ADEQUACY	3.14	2	.2079	-34.1366
TOXICITY	1.00	1	.3176	-34.6360
MUTAGENICITY	.19	2	.9081	-34.2330

AVERAGE SLOPE (NONLIN. MODEL) = .072
95% CONF. LIMITS = (.000, 209.011)

AVERAGE SLOPE (LINEAR REGR.) = -.021
95% CONF. LIMITS = (-.139, .098)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 03/30/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA98

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL									
2-NF	-	3.00	300	312	315			309.00	7.94
2-AA	RLA026	0.50	575	837	853			855.00	19.08
NEG CONTROL									
DIMETHYLSULF	RLA026	100.000	60	41	48			49.67	9.61
	-	100.000	28	33	30			30.33	2.52
BMGS-84-0003									
	RLA026	1.00	49	32				40.50	12.02
	RLA026	5.00	48	48				48.00	0.00
	RLA026	10.00	45	37				41.00	5.66
	RLA026	30.00	37	50				43.50	9.19
	RLA026	50.00	43	61				52.00	12.73
	RLA026	100.00	45	43				44.00	1.41
	RLA026	300.00	37	22				29.50	10.61
	RLA026	500.00	32	35				33.50	2.12
	RLA026	1000.00	36	35				35.50	0.71
	-	1.00	21	26				23.50	3.54
	-	5.00	26	25				25.50	0.71
	-	10.00	25	28				26.50	2.12
	-	30.00	21	24				22.50	2.12
	-	50.00	22	30				26.00	5.66
	-	100.00	17	24				20.50	4.95
	-	300.00	43	27				35.00	11.31
	-	500.00	20	26				23.00	4.24
	-	1000.00	23	25				24.00	1.41

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T*-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMCS-84-0003 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	60	41	48	49.67	9.61
.50*	UGS	875	837	853	855.00	19.08
1.00	UGS	49	32		40.50	12.02
5.00	UGS	48	48		48.00	.00
10.00	UGS	45	37		41.00	5.66
30.00	UGS	37	50		43.50	9.19
50.00	UGS	43	61		52.00	12.73
100.00	UGS	45	43		44.00	1.41
300.00	UGS	37	22		29.50	10.61

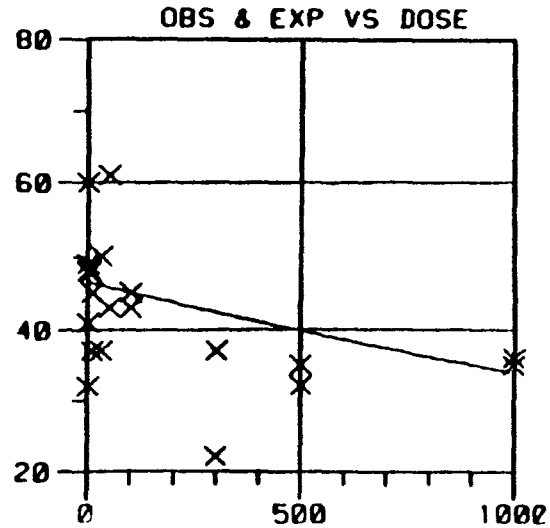
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	46.502	-13.8794	1.3284	.00032

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	17.13	11	.1040	-66.9456
ADEQUACY	16.44	6	.0116	-75.1647
TOXICITY	8.17	1	.0043	-79.2493
MUTAGENICITY	.01	2	.9950	-74.1945

AVERAGE SLOPE (NONLIN. MODEL) = .000
95% CONF. LIMITS = (.000,********)

AVERAGE SLOPE (LINEAR REGR.) = -.504
95% CONF. LIMITS = (-1.914, .906)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: -
STRAIN: TA98 DATE: 03/30/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	28	33	30	30.33	2.52
3.00*	UGS	300	312	315	309.00	7.94
1.00	UGS	21	26		23.50	3.54
5.00	UGS	26	25		25.50	.71
10.00	UGS	25	28		26.50	2.12
30.00	UGS	21	24		22.50	2.12
50.00	UGS	22	30		26.00	5.66
100.00	UGS	17	24		20.50	4.95
300.00	UGS	43	27		35.00	11.31

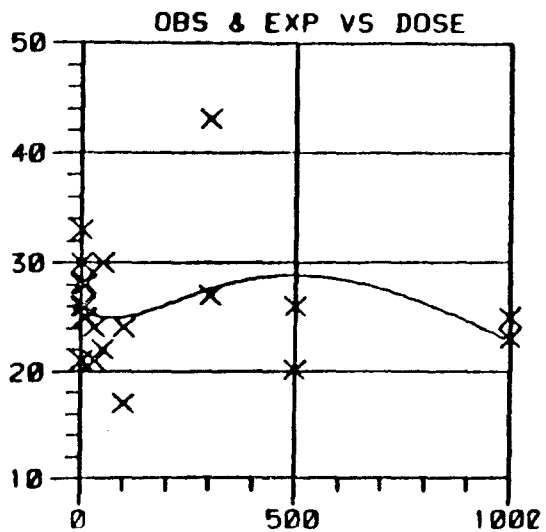
MORE THAN 9 DOSE LEVELS USED IN COMPUTATION BUT NOT DISPLAYED

	B(0)	B(1)	B(2)	B(3)
ESTS.	26.262	-5.6194	1.5624	.00218

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	8.29	11	.6873	-57.5101
ADEQUACY	11.20	6	.0823	-63.1109
TOXICITY	1.73	1	.1890	-63.9737
MUTAGENICITY	1.40	2	.4956	-63.8130

AVERAGE SLOPE (NONLIN. MODEL) = .090
95% CONF. LIMITS = (.013, .620)

AVERAGE SLOPE (LINEAR REGR.) = .024
95% CONF. LIMITS = (- 004, .052)



MUTAGENICITY TESTING OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

IN VITRO ASSAYS WITH SALMONELLA TYPHIMURIUM
OF ARMY DYE PURIFIED YELLOW
RESEARCH LAB: GBBA ON 04/06/84 08/27/84

TEST TYPE: STANDARD PLATE INCORPORATION STRAIN: TA98

COMPOUND	ACT	UGS PER PLATE	HISTIDINE REVERTANTS PER PLATE A	B	C	D	E	MEAN	STD
POS CONTROL 2-NF	RLA026	0.50	740	825	817			794.00	46.94
	-	3.00	250	270	255			258.33	10.41
NEG CONTROL DIMETHYLSULF	RLA026	100.000	42	31	43			38.67	6.66
	-	100.000	23	29	20			24.00	4.58
BMGS-84-0003									
	RLA026	1.00	24	39				31.50	10.61
	RLA026	5.00	42	42				42.00	0.00
	RLA026	10.00	52	49				50.50	2.12
	RLA026	30.00	50	74				62.00	16.97
	RLA026	50.00	52	54				53.00	1.41
	RLA026	100.00	63	62				62.50	0.71
	RLA026	300.00	36	32				34.00	2.83
	-	1.00	37	28				32.50	6.36
	-	5.00	29	20				24.50	6.36
	-	10.00	28	24				26.00	2.83
	-	30.00	39	18				28.50	14.85
	-	50.00	27	40				33.50	9.19
	-	100.00	20	21				20.50	0.71
	-	300.00	28	32				30.00	2.83

PHENOCOPY CHECK : TRUE MUTANTS
STERILITY S-9 : NOT CONTAMINATED
SAMPLE STERILITY: NOT CONTAMINATED
ACT MIX/PLATE : 500UGS

T=-TOXIC
TNTC-TOO NUMEROUS TO COUNT
NATC-NOT ABLE TO COUNT

G-PGS T-PPT
N-NGS P-PPM
M-MGS B-PPB
L-NLS I-MM
U-ULS C-UM

STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GBBA ACTIVATION: + RLA026
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

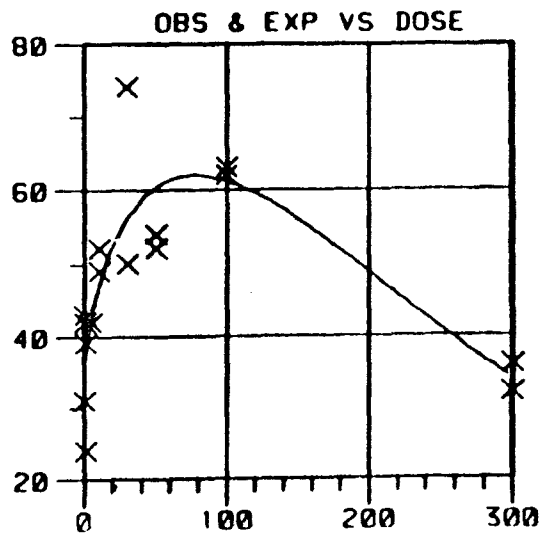
DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	42	31	43	38.67	6.66
.50*	UGS	740	825	817	794.00	46.94
1.00	UGS	24	39		31.50	10.61
5.00	UGS	42	42		42.00	.00
10.00	UGS	52	49		50.50	2.12
30.00	UGS	50	74		62.00	16.97
50.00	UGS	52	54		53.00	1.41
100.00	UGS	63	62		62.50	.71
300.00	UGS	36	32		34.00	2.83

	B(0)	B(1)	B(2)	B(3)
ESTS.	35.875	.9542	.7319	.00600

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	10.88	9	.2840	-53.4546
ADEQUACY	6.82	4	.1459	-56.8628
TOXICITY	30.69	1	.0000	-72.2075
MUTAGENICITY	37.36	2	.0000	-75.5451

AVERAGE SLOPE (NONLIN. MODEL) = .756
95% CONF. LIMITS = (.240, 2.381)

AVERAGE SLOPE (LINEAR REGR.) = .251
95% CONF. LIMITS = (.104, .399)



STATISTICAL ANALYSIS: MUTAGENICITY OF PURIFIED YELLOW DYE IN SALMONELLA TYPHIMURIUM

SAMPLE ID: BMGS-84-0003 LAB: GB6A ACTIVATION: -
STRAIN: TA98 DATE: 04/06/84 TECHNICIAN: MJK

DOSE	UNITS	PLATE COUNTS			MEAN	S.D.
.00	UGS	23	29	20	24.00	4.58
3.00*	UGS	250	270	255	258.33	10.41
1.00	UGS	37	28		32.50	6.36
5.00	UGS	29	20		24.50	6.36
10.00	UGS	28	24		26.00	2.83
30.00	UGS	39	18		28.50	14.85
50.00	UGS	27	40		33.50	9.19
100.00	UGS	20	21		20.50	.71
300.00	UGS	28	32		30.00	2.83

	B(0)	B(1)	B(2)
ESTS.	24.000	1.7260	.0000

NO EVIDENCE OF TOXICITY

TEST	CHI-SQUARE	DF	P	LOGL
POISSON	15.51	9	.0779	-51.3718
ADEQUACY	10.72	5	.0573	-56.7301
MUTAGENICITY	.07	2	.9650	-56.7657

AVERAGE SLOPE (NONLIN. MODEL) = .019
95% CONF. LIMITS = (.004, .089)

AVERAGE SLOPE (LINEAR REGR.) = .007
95% CONF. LIMITS = (-.030, .043)